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(54) Title: COMPOSITIONS AND METHODS FOR THE IDENTIFICATION AND QUANTITATION OF DELETION SEQUENCE OLIGONUCLEOTIDES IN SYNTHETIC OLIGONUCLEOTIDE PREPARATIONS

(57) Abstract

The invention relates to compositions and methods for the identification and quantitation of a mixture of various deletion sequence oligonucleotides present in a preparation of a synthetic oligonucleotides of length n via hybridization reactions.

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COMPOSITIONS AND METHODS FOR THE IDENTIFICATION AND QUANTITATION OF DELETION SEQUENCE OLIGONUCLEOTIDES IN SYNTHETIC OLIGONUCLEOTIDE PREPARATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a Continuation-In-Part of U.S. patent application Serial No. 08/923,771, filed September 2, 1997, the entire contents of which are hereby incorporated by reference.

FIELD OF THE INVENTION

The present invention relates to compositions and methods for the identification and quantitation of a mixture of various deletion sequence oligonucleotides present in a preparation of a synthetic oligonucleotide of length *n* via hybridization reactions. Unlike chromatographic methods, the hybridization reactions of the invention take place in the absence of electrophoresis or any other flow process and are

allowed to proceed to equilibrium. The invention may be used to quantitate the deletion sequence oligonucleotide species present in synthetic preparations of a variety of oligonucleotides, as well as preparations of any molecule which is not technically an oligonucleotide but which has a nucleobase sequence and is capable of hybridizing to a nucleic acid (e.g., peptide nucleic acids). Although any mixture of deletion sequence oligonucleotides may be reliably quantitated in a cost-effective manner by the compositions and methods of the invention, a preferred embodiment is drawn to the characterization of a mixture of (n-1)-mers present in a synthetic oligonucleotide preparation (i.e., a set of deletion sequence oligonucleotides that have different nucleobase sequences apparently resulting, in each instance, from the deletion of a single base from an oligonucleotide having a nucleobase sequence of length n).

BACKGROUND OF THE INVENTION

During a typical oligonucleotide synthesis, nucleoside monomers are attached to a growing oligomer chain one at a time in a repeated series of chemical reactions such as nucleoside monomer coupling, oxidation, capping and detritylation. The stepwise yield for each nucleoside addition is above 99%. Although impressive, such a yield indicates that some amount (less than 1%) of the preparation of the oligomer chain has failed at each nucleoside monomer addition cycle (Smith, *Anal. Chem.*, 1988, *60*, 381A). Thus,

the final yield of full-length oligonucleotide is not 100% in a synthetic preparation thereof and decreases as *n* (the number of nucleobases in the full-length oligonucleotide) increases.

Oligonucleotides shorter than the full-length (*n* bases) oligonucleotide, ranging from (*n*-1)-, (*n*-2)-, *etc.*, to 1-mers (nucleotides), become present as possibly undesirable impurities in the *n*-mer olignucleotide product. Among the impurities, (*n*-2)-mer and shorter oligonucleotide impurities are typically present in very small amounts and can be easily removed by chromatographic purification (Warren *et al.*, Chapter 9 *In: Methods in Molecular Biology, Vol. 26: Protocols for Oligonucleotide Conjugates*, Agrawal, S., Ed., 1994, Humana Press Inc., Totowa, NJ, pages 233-264).

However, due to the lack of chromatographic selectivity and product yield, the (*n*-1)-mer impurities are usually still present in the full-length oligonucleotide (*n*-mer) product after the purification process, unless a very low yield of desired *n*-mer is acceptable. It is known in the art to purify oligonucleotides by denaturing polyacrylamide gel electrophoresis (PAGE), but such methods are not applicable to the mass production of oligonucleotides as the yields obtained by such methods are typically less than 50% (Ausubel *et al.*, eds., *Short Protocols in Molecular Biology*, 2nd Ed., Greene Publishing Associates and John Wiley & Sons, New York, 1992, pages 2-33 to 2-38). Therefore, after chromatographic

purification, the total amount of (n-1)-mer impurities in the n-mer oligonucleotide product is often only about a few percent. The (n-1) portion likely consists of the mixture of all possible single base deletion sequences relative to the n-mer parent oligonucleotide, although some reports suggest otherwise (see, e.g., Temsamani *et al.*, *Nucleic Acids Research*, 1995, *23*, 1841).

Such (n-1) impurities can be classified as terminal deletion or internal deletion sequences, depending upon the position of the missing base, *i.e.*, either at the 5' or 3' terminus or internally. When an oligonucleotide containing single terminal base deletion sequence impurities is used as a drug (Crooke, *Hematologic Pathology*, 1995, *9*, 59), the terminal deletion sequence impurities will usually bind to the same target mRNA as the full length sequence but with a slightly lower affinity. Thus, to some extent, such impurities can be considered as part of the active drug component.

However, the internal single base deletion sequence impurities are not expected to hybridize well to the target mRNA and thus will have either little to no biological activity or undesired biological activity. There are potential side effects for the internal single base deletion sequence impurities, including the chance that some of the internal single base deletion sequence impurities would be complementary to a non-target mRNA, leading to an unintended

biological response. Therefore, the speciation of the single base deletion sequence impurities, particularly the internal ones, is a parameter of the impurity profile of oligonucleotide drugs (Crooke, *Antisense Research and Development*, 1993, *3*, 301-306).

Some attempts have been made to develop a reliable, inexpensive means of quantitating (*n*-1) oligonucleotide impurities. Anion exchange high pressure liquid chromatography (HPLC) can separate full length phosphodiester oligonucleotide from their deletion sequences (Agrawal *et al.*, *J. Chromatography*, 1990, 509, 396). However, the resolution is decreased considerably for chemically modified oligonucleotides, such as phosphorothioates, which may be preferred for therapeutic or pharmaceutical uses (Warren *et al.*, Chapter 9 *In: Methods in Molecular Biology, Vol. 26: Protocols for Oligonucleotide Conjugates*, Agrawal, S., Ed., 1994, Humana Press Inc., Totowa, NJ, pages 233-264). Reversed phase HPLC does not seem to be able to discriminate and resolve the spectrum of full length oligonucleotides and their deletion sequence impurities (Gelfi *et al.*, *Antisense Nucleic Acid Drug Dev.*, 1996, 6, 47-53). Capillary gel electrophoresis (CGE) has excellent resolving power for oligonucleotides and can separate (*n*-1)-mer impurities from the *n*-mer product with acceptable resolution (Srivatsa, *J. Chromatogr. A*, 1994, *680*, 469; DeDionisio *et al.*, 1996, *735*,

191; Gelfi *et al.*, *Antisense Nucleic Acid Drug Dev.*, 1996, *6*, 47; Cohen *et al.*, *Adv. Chromatography*, 1996, *37*, 127). However, all of the (*n*-1)-mers migrate at the same speed and therefore are detected as a single peak in capillary electrophoresis.

To date, only a few reports have addressed the issue of sequence identity of the (*n*-1) impurities. Electrospray ionization mass spectrometry (ES/MS) has been employed to analyze the (*n*-1) impurities of a phosphorothioate oligonucleotide (Fearon *et al.*, *Nucleic Acids Research*, 1995, *23*, 2754; Cohen *et al.*, *Adv. Chromatography*, 1996, *37*, 127). There are, however, several drawbacks associated with the method: (1) ES/MS can only detect peaks corresponding to (*n*-1)-mers with different nucleosides missing, that is, it cannot distinguish the (*n*-1)-mers with the same nucleotide missing at different positions of the parent oligonucleotide; (2) the resolution between the (*n*-1)-mer peaks is usually inadequate for quantitation; and (3) even in those instances where the resolution is acceptable, the signal to noise ratio of the peaks is not high enough to ensure accurate quantitation. For all these reasons, the electrospray mass spectrometry method has been limited to the identification of the full length oligonucleotide (Bayer *et al.*, *Anal. Chem.*, 1994, *66*, 3858), or for the characterization of the purity of oligonucleotides (Deroussent *et al.*, *Rapid Commun. Mass Spectrom.*, 1995, *9*, 1).

A semi-biological method for identification of the (*n*-1)-mer oligonucleotide population has also been reported (Temsamani *et al.*, *Nucleic Acids Research*, 1995, *23*, 1841). In this method, the oligonucleotides were tailed with poly-dA (12-20 residues) and annealed to a dT-tailed plasmid. The recombinant plasmid was ligated and used to transform competent bacteria. Clones were randomly selected and the region in the recombinant plasmid containing the inserted oligonucleotide was sequenced. A possible problem to this method is that the plasmid and its host bacteria might be biased towards or against the selection of inserts having a particular sequence; therefore, the results arrived at after cloning may be different from the actual distribution of the (*n*-1) population. The complicated procedure and tedious labor also make the method less attractive; hundreds of clones would have to be prepared and sequenced in order to get meaningful statistical information regarding the (*n*-1) population. In addition, this method is limited to ligatable, clonable oligonucleotides, *i.e.*, phosphodiester oligonucleotides, and might not be adaptable to oligonucleotides having one or more synthetic chemical alterations.

The present invention surmounts these and other limitations. The compositions and methods of the invention provide the means to distinguish deletion sequence oligonucleotides having related but different nucleobase sequences and to quantitate the amounts of different species of deletion sequence ("target") oligonucleotides present in a

mixture thereof. Such mixtures include, but are not limited to, solutions containing a set of (n-1)-mers with a nucleobase (e.g., A, G, C, m5C, T or U) missing at different positions of the full-length (n) oligonucleotide's sequence. Furthermore, the invention is equally applicable to oligodeoxynucleotides as well as oligonucleotides having synthetic chemical alterations, so long as such alterations do not modify the specificity of the oligonucleotide's nucleobase sequence for its reverse complement.

BRIEF SUMMARY OF THE INVENTION

The present invention relates to compositions and methods for the identification and quantitation of a mixture of various deletion sequence oligonucleotides present in a preparation of a synthetic oligonucleotide of length n. During the synthesis of a full-length oligonucleotide (n-mer), various deletion oligonucleotides [in particular, (n-1)-mers] are also generated. Such deletion oligonucleotides may have a variety of nucleobase sequences related to the base sequence of the full-length oligonucleotide.

In the methods of the invention, a solution comprising a mixture of various deletion sequence oligonucleotides that have been detectably labeled is contacted to a composition comprising a series of immobilized probe oligonucleotides. For example, a mixture of (n-1) deletion sequence oligonucleotides having differing sequences is hybridized to a

composition comprising a variety of probe oligonucleotides, each probe oligonucleotide having a nucleobase sequence that is the precise reverse complement of a given (n-1) deletion sequence oligonucleotide, wherein a reverse complement probe oligonucleotide is present for every possible (n-1)-mer that can be present in a preparation of a synthetic oligonucleotide of length n having a defined nucleobase sequence. The hybridization reaction is conducted under conditions such that each particular (n-1) species is allowed to hybridize (bind) specifically, and with high affinity, to its appropriate reverse complement probe. In particular, the hybridization reactions are allowed to proceed for a relatively extended period of time in the absence of flow and under other such conditions as are necessary to allow the hybridization reactions to proceed to equilibrium.

Unbound oligonucleotides (including, for example, n-mers) may then be removed from the hybridization reaction by washing. Relative or absolute concentrations of the various hybridized (bound) n-1 deletion sequence oligonucleotides is determined using any of a variety of means. The relative or absolute concentration of each (n-1)-mer in the preparation of the full-length synthetic oligonucleotide of length n, from which the sample was taken, is then calculated using this data.

RELATED ART

U.S. Patent 5,429,807 to Matson *et al.* claims devices and methods for synthesizing biopolymers, including polynucleotides, on two-dimensional surfaces.

U.S. Patent 5,436,327 to Southern *et al.* claims a method of synthesizing an immobilized oligonucleotide.

U.S. Patent 5,632,957 to Heller *et al.* is stated to describe a system for performing molecular diagnoses, including the identification of point mutations in DNA samples. An electrophoretic field is required in the methods of Heller *et al.* (see column 8, lines 18-40 and column 9, lines 24-28).

U.S. Patent 5,700,637 to Southern is stated to describe an apparatus and method for analyzing a polynucleotide sequence.

Published PCT patent application WO 98/31836 describes methods, compositions and algorithms for the detection and quantification of nucleic acid species. The methods and compositions of Drmanac are stated to be useful in techniques such as, for example, sequencing by hybridization and detection of nucleic acids from infectious agents.

Published PCT patent application WO 98/11210 describes compositions and methods for target nucleic acid detection using a composition which has two adjacent ligatable polynucleotides attached to a solid phase. When a nucleic acid that hybridizes to both attached ligatable polynucleotides is contacted with the composition, the termini of the two ligatable polynucleotides are brought into contact

with each other. If ligase is then added, a single "looped" polynucleotide attached to the solid phase is formed from the ligation of the two ligatable polynucleotides.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

This invention provides new methods and compositions for the identification and quantitation of deletion sequence oligonucleotides. The invention is capable of distinguishing and quantitating a mixture having from three (3) to about fifty (50) oligonucleotides of the same or similar length, each oligonucleotide having a nucleobase sequence that represents a deletion of one or more bases from the sequence of a parent oligonucleotide. The invention is distinct from, and required solutions to a distinct set of technical problems from those found in the development of, e.g., methods and compositions that can distinguish between two, or at most a few, nucleic acids having single base mismatches relative to each other (see, e.g., Wallace *et al.*, *Nucleic Acids Research*, 1979, *6*, 3543).

The invention may be used to quantitate the deletion sequence oligonucleotide species present in synthetic preparations of a variety of oligonucleotides, as well as preparations of any molecule which is not technically an oligonucleotide but which has a nucleobase sequence and is capable of hybridizing to a nucleic acid (e.g., peptide nucleic acids). In a preferred embodiment, the methods and compositions of the invention are used to characterize the

types, and quantitate the amounts, of different (*n*-1)-mers present in a sample from a preparation of synthetic full length oligonucleotides (*n*-mers).

The methods of the invention preferably comprise up to four steps. For example, as applied to the characterization of (*n*-1)-mers, step (a) comprises isolating a representative sample of the (*n*-1) portion of an oligonucleotide preparation, and labeling all or a substantial portion of the (*n*-1)-mers in the sample. Step (b) comprises contacting a mixture comprising the labeled "target" (*n*-1) deletion sequence oligonucleotides with a composition (which may be a matrix) comprising from 2 to about 50 sensor arrays of the invention. Under appropriate conditions, the hybridization reactions between each target (*n*-1) oligonucleotide and its corresponding "probe" oligonucleotide on a sensor array display essentially absolute hybridization specificity (Wallace *et al.*, *Biochimie*, 1985, 67, 755). Applicants have discovered that, for particularly accurate determinations, allowing the hybridization reactions to proceed to equilibrium is preferred. Optional step (c) comprises washing the matrix to remove any unhybridized material (e.g., mismatched and/or unmatched oligonucleotides). Step (d) comprises detecting and quantitating labeled target oligonucleotides bound to the sensor arrays.

The compositions of the invention comprise a series of

sensor arrays, each of which comprises a "probe" oligonucleotide having a unique nucleobase sequence. Each sensor array preferably comprises up to four parts. Part (1) provides a solid support for the other parts (e.g., a glass slide). Part (2) is a first linker (e.g., a hexylamino group) that attaches one or more of the other parts to the solid support. Part (3) is an optional second linker, or spacer unit, to distance the "probe" oligonucleotide from the solid support. Part (4) is a "probe" oligonucleotide which has a nucleotide sequence that is the reverse complement of that of a unique deletion sequence oligonucleotide. Typically, a series of sensor arrays, each of which comprises a probe oligonucleotide having a different sequence than those of the other sensor arrays, is attached to a common (shared) solid support, although other arrangements can be used. In a preferred embodiment, the sensor arrays are arranged in a matrix on a shared solid support.

Each "probe" oligonucleotide has a nucleobase sequence that is the precise reverse complement of a corresponding "target" deletion sequence oligonucleotide and is thus capable of specific hybridization with a unique deletion sequence oligonucleotide species. Under appropriate conditions, each target sequence deletion oligonucleotide hybridizes with (binds to) specifically to its corresponding probe oligonucleotide. Applicants have discovered that, for particularly accurate determinations, allowing the hybridization reactions to proceed to equilibrium is

preferred. The amount of bound labeled target oligonucleotide bound to a particular sensor array, which correlates directly with the amount of the corresponding deletion sequence oligonucleotide present in the sample, is then determined.

EXEMPLARY METHODS OF THE INVENTION

Step (a): First, a sample solution containing a mixture of deletion sequence oligonucleotides is isolated by means known in the art or explained herein. For example, in one embodiment of the invention, the sample solution containing full length oligonucleotide and all of the (*n*-1) deletion sequences is loaded onto a polyacrylamide slab gel, and a solution containing primarily or only (*n*-1) components is obtained by electrophoretic separation and subsequent electroelution of the (*n*-1) band from the gel. Alternatively, HPLC or CGE can be used to isolate a sample of (*n*-1)-mers. For quantitative determinations, the relative or absolute amounts of [total deletion sequence oligonucleotides] and [full length oligonucleotide] are determined, either at the same time the sample solution is isolated or by an independent method.

Next, the deletion sequence oligonucleotides in the mixture are detectably labeled with, e.g., an enzyme, a fluorescent dye or a radioisotope (e.g., biotin-streptavidin, fluorescein isothiocyanate, ^{35}S, ^{32}P or the like). As will be appreciated by those skilled in the art, to avoid biased

results, an approximately equivalent amount of label should be associated with or incorporated into each deletion sequence oligonucleotide.

Step(b): The mixture of labeled deletion sequence oligonucleotides is contacted to a composition according to the invention. The composition, which may be in the form of a matrix, comprises a plurality of sensor arrays, wherein each sensor array comprises a unique oligonucleotide probe that is complementary to only one of the potential deletion sequence oligonucleotides. Each oligonucleotide probe forms a match with its corresponding deletion sequence oligonucleotide and a mismatch with other deletion sequence oligonucleotides present in the mixture. Under appropriately stringent hybridization conditions, i.e., conditions under which the hybridization (binding) of complementary oligonucleotide is preferentially achieved, mismatched oligonucleotides hybridize poorly or not at all to the compositions of the invention. The hybridization reactions take place in the absence of electrophoresis or any other flow and are allowed to proceed to equilibrium.

The degree of hybridization between the individual probes of the sensor array and the complementary (n-1) oligonucleotides is dependent upon parameters such as the ionic strength of the buffer solution in which the hybridization occurs, temperature, base composition and length of the duplex formed between the target oligonucleotide and the sensor array, concentration of the sensor array,

concentration of the target oligonucleotide, and the concentration(s) of duplex destabilizing agent(s). The method of the invention is designed to maximize the affinity of the probes of the sensor array for the target oligonucleotide while achieving the least degree of affinity for other (n-1) oligonucleotides in the mixture.

The following serve as examples of the buffer solutions that can be applied; others will be apparent to those skilled in the art:

(1) SSPE buffer (1x-5x) and 0.1-0.5% SDS; 5x SSPE buffer is 0.75 M NaCl, 50 mM NaH_2PO_4, pH 7.4, and 5 mM EDTA;

(2) 0.9 M NaCl, 5 mM EDTA, 90 mM Tris-HCl, pH 7.2-7.6, 0.1-0.5% SDS;

and

(3) 50% Formamide, 5x SSPE , 0.1 % SDS, 10% dextran sulfate.

Temperature can be another important parameter for hybridization reactions. In the methods of the invention, the temperature of the hybridization reaction is adjusted so that only the target oligonucleotide will quantitatively hybridize to the sensor array. At optimum temperatures, the formation of duplexes between the sensor array and undesired oligonucleotides will be thermodynamically disfavored. As is known in the art, optimum temperatures can be estimated from

the melting temperature (T_m), the temperature at which 50% of the duplex dissociates.

In general, the temperature for hybridization reactions should be between the melting temperature of the target oligonucleotide duplex, T_m^d, and the highest melting temperature of undesired deletion sequence oligonucleotides, T_m^u ("*d*" for desired and "*u*" for undesired). That is, the temperature range at which the hybridization reaction is performed, T, is defined by the equation

$$T_m^u < T < T_m^d.$$

Methods for estimating and determining these parameters are known in the art (see, for example, Lehninger, *Biochemistry*, 2nd Ed., *1970*, Worth Publishers Inc., New York, NY, page 875; Jarrett, *J. Chromatogr.*, 1993, *618*, 315; Freier, Chapter 5 *In: Antisense Research and Applications*, Crooke *et al.*, Eds., *1993*, CRC Press, Boca Raton, LA, pages 67-82). In some embodiments of the invention, the temperature at which the hybridization reactions of step **(b)** occur can be increased so that unmatched and/or mismatched oligonucleotides do not hybridize as well, or at all, to the probe oligonucleotides of the sensor arrays.

In a preferred embodiment of the invention, the hybridization reactions take place in the absence of

electrophoresis or any other flow and are allowed to proceed to equilibrium. Applicants have discovered that, for particularly accurate determinations, allowing the hybridization reactions to proceed to equilibrium is preferred.

The methods of the invention optionally comprise the addition of unlabeled target (*n*-1) oligonucleotides during the hybridization reactions. The presence of unlabeled (*n*-1)-mers serves to enhance the hybridization specificity of the probe oligonucleotides of the sensor arrays for their corresponding labeled target oligonucleotides.

Step (c): In this optional, but preferred, step, unbound oligonucleotides are removed by washing. Removal of mismatched (undesired) target oligonucleotides, is achieved by placing the hybridized probe:target oligonucleotide complexes into a suitable washing buffer which has a composition that is similar, or even identical, to that of the hybridization buffer of step (b) but which is different in concentration. When identical in composition to the hybridization buffer, the washing buffer can be from 0.4x to 2x, preferably from 0.5x to 1.6x, and most preferably from 0.6x to 1.2x the concentration of the hybridization buffer. By way of example, if the hybridization buffer is 3x SSPE buffer, the washing buffer is from 1.2x to 6x SSPE buffer, preferably from 1.5x to 4.8x SSPE buffer, and most preferably from 1.8x to 3.6x SSPE buffer. The purpose of the optional washing step is to remove as much unbound target deletion sequence oligonucleotide molecules as

possible while maintaining the highest possible concentration of target oligonucleotide bound to the sensor arrays. Accordingly, the specific conditions at which these steps are carried out may be adjusted by monitoring these parameters.

Step (d): In this step, the signal intensity of bound label for each sensor array is determined using any appropriate means. For example, radiolabeled oligonucleotides are detected by autoradiography or radiodensitometry, and fluorescently labeled oligonucleotides are detected by measuring the fluorescence present at a given sensor array. Enzymatically labeled oligonucleotides are detected by adding a substrate that undergoes a detectable change (e.g., a chromogenic reaction) that results from the presence of the enzyme. Regardless of the detection means used, the signal intensity is directly proportional to the amount of the specific labeled deletion sequence oligonucleotide bound to a specific sensor array via its particular reverse complementary oligonucleotide probe.

The relative or absolute amount of each deletion sequence oligonucleotide in the sample solution is determined by comparing the signal intensities of the various sensor arrays. The relative amount of a given deletion sequence oligonucleotide in the sample is multiplied by the total concentration of deletion sequence oligonucleotides in the preparation to yield the absolute concentration of that particular deletion sequence oligonucleotide in the synthetic preparation.

Exemplary Characterization of (n-1)-mers

In a preferred embodiment, the methods of the invention are used to characterize a mixture of (n-1) deletion sequence oligonucleotides present in a preparation of synthetic oligonucleotide of length n. Such a mixture consists of a set of oligonucleotides, all of which have a length of (n-1) nucleobases, but each of which has a different nucleobase sequence resulting, in each instance, from the deletion of a single base from the nucleobase sequence of the full-length oligonucleotide. Although methods are known for estimating the amount of total (n-1)-mer present in a sample, there has been no reliable and cost-effective means of accurately determining the amounts of various (n-1)-mers having different base sequences prior to the present invention.

In this embodiment of the invention, a mixture of (n-1) deletion sequence oligonucleotides having differing sequences is hybridized to a composition comprising a variety of probe oligonucleotides. Each probe consists essentially of an oligonucleotide having a nucleobase sequence that is the precise reverse complement of a given (n-1) deletion sequence oligonucleotide, and a reverse complement probe oligonucleotide is present for every possible (n-1)-mer that can be present in a preparation of a synthetic oligonucleotide of length n having a given nucleobase sequence. The end product of this embodiment of the invention is a determination

of the relative and absolute amounts of each (n-1) species present in the preparation of a synthetic oligonucleotide. Such information is useful, for example, for characterizing the contaminating (n-1) oligonucleotide species that may be present in a preparation of an oligonucleotide intended for therapeutic use.

Exemplary Compositions of the Invention

The compositions of the invention comprise a solid support (1) to which a plurality of sensor arrays is attached. Each sensor array comprises up to three parts: a first linker to the solid support (2), an optional second linker or spacer (3) and an oligonucleotide probe (4) having a sequence that is the reverse complement of a target deletion sequence oligonucleotide which is a member of a mixture of such target oligonucleotides. The linker (2) and, optionally, the spacer (3) provide a bridge between the solid support (1) and the oligonucleotide probe (4) in such a way as to not significantly alter or reduce the binding capacity of the latter element. The following sections describe these elements in more detail.

Exemplary solid supports (1) include, but are not limited to, graft polymers (U.S. Patent No. 4,908,405 to Bayer and Rapp); polyacrylamide (Fahy *et al.*, *Nucl. Acids Res.*, 1993, *21*, 1819); polyacrylmorpholide, polystyrene and derivatized

polystyrene resins (Syvanen *et al.*, *Nucl. Acids Res.*, 1988, *16*, 11327; U.S. Patent Nos. 4,373,071 and 4,401,796 to Itakura), including amino methyl styrene resins (U.S. Patent No. 4,507,433 to Miller and Ts'O); copolymers of N-vinylpyrrolidone and vinylacetate (Selinger *et al.*, *Tetrahedron Letts.*, 1973, *31*, 2911; Seliger *et al.*, *Die Makromolekulare Chemie*, 1975, *176*, 609; and Selinger, *Die Makromolekulare Chemie*, 1975, *176*, 1611); TEFLON™ (Lohrmann *et al.*, *DNA*, 1984, *3*, 122; Duncan *et al.*, *Anal. Biochem.*, 1988, *169*, 104); controlled pore glass (Chow *et al.*, *Anal. Biochem.*, 1988, *175*, 63); polysaccharide supports such as agarose (Kadonaga, *Methods Enzymol.*, 1991, *208*, 10; Arndt-Jovin *et al.*, *Eur. J. Biochem.*, 1975, *54*, 411; Wu *et al.*, *Science*, 1987, *238*, 1247; Blank *et al.*, *Nucleic Acids Res.*, 1988, *16*, 10283) or cellulose (Goldkorn *et al.*, *Nucl. Acids Res.*, 1986, *14*, 9171; Alberts *et al.*, *Meth. Enzymol.*, 1971, *21*, 198) or derivatives thereof, *e.g.*, DEAE-cellulose (Schott, *J. Chromatogr.*, 1975, *115*, 461) or phosphocellulose (Siddell, *Eur. J. Biochem.*, 1978, *92*, 621; Bunemann *et al.*, *Nucl. Acids Res.*, 1982, *10*, 7163; Noyes *et al.*, *Cell*, 1975, *5*, 301; Bunemann *et al.*, *Nucl. Acids Res.*, 1982, *10*, 7181); dextran sulfate (Gingeras *et al.*, *Nucl. Acids Res.*, 1987, *15*, 5373); polypropylene (Matson *et al.*, *Anal. Biochem.*, 1994, *217*, 306);

agarose beads (Kadonaga *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 1986, *83*, 5889); latex particles (Kawaguchi *et al.*, *Nucleic Acids Res.*, 1989, *17*, 6229); nylon beads (Van Ness *et al.*, *Nucl. Acids Res.*, 1991, *19*, 3345); paramagnetic beads (Gabrielson *et al.*, *Nucl. Acids Res.*, 1989, *17*, 6253; Lund, et al., *Nucl. Acids Res.*, 1988, *16*, 10861; Day *et al.*, *Biochem. J.*, 1991, *278*, 735); silica gels (Yashima *et al.*, *J. Chromatogr.*, 1992, *603*, 111); derivatized forms of silica gels, polytetrafluoroethylene, cellulose or metallic oxides (U.S. Patent No. 4,812,512 to Buendia); and art-recognized equivalents of any of the preceding solid supports; microtiter plates (Drmanac *et al.*, *Science*, 1993, *260*, 1649); crosslinked copolymers of N-vinylpyrrolidone, other N-vinyl-lactam monomers and an ethylenically unsaturated monomer having at least one amine or amine-displacable functionality as disclosed in U.S. Patent No. 5,391,667. In one set of preferred embodiments, polystyrene or long chain alkyl CPG (controlled pore glass) beads are employed. In another set of preferred embodiments, microscopic glass slides are employed (Fodor *et al.*, *Science*, 1991, *251*, 767; Maskos *et al.*, *Nucleic Acids Research*, 1992, *20*, 1679; Guo *et al.*, 1994, *22*, 5456; Pease *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 1994, *91*, 5022).

The first linker (2) may be selected from a variety of chemical linking groups or chains. Any chemical group or chain capable of forming a chemical linkage between the solid

support (1) and the probe oligonucleotide (4) [or, if it is present, the optional spacer (3)] may be employed. A suitable linker has the preferred characteristic of non-reactivity with compounds introduced during the various steps of oligonucleotide synthesis. It will be appreciated by those skilled in the art that the chemical composition of the solid support (1), the probe oligonucleotide (4) and, if present, the optional spacer (3) will influence the choice of the linker (2).

Many suitable linkers will comprise a primary amine group at either or both termini, as many chemical reactions are known in the art for linking primary amine groups to a variety of other chemical groups; however, other terminal reactive moieties are known and may be used in the invention. Suitable linkers include, but are not limited to, linkers having a terminal thiol group for introducing a disulfide linkages to the solid support (Day *et al.*, *Biochem. J.*, 1991, *278*, 735; Zuckermann *et al.*, *Nucl. Acids Res.*, *15*, 5305); linkers having a terminal bromoacetyl group for introducing a thiol-bromoacetyl linkage to the solid support (Fahy *et al.*, *Nucl. Acids Res.*, 1993, *21*, 1819); linkers having a terminal amino group which can be reacted with an activated 5' phosphate of an oligonucleotide (Takeda *et al.*, *Tetrahedron Letts.*, 1983, *24*, 245; Smith *et al.*, *Nucl. Acids Res.*, 1985, *13*, 2399; Zarytova *et al.*, *Anal. Biochem.*, 1990, *188*, 214);

poly(ethyleneimine) (Van Ness *et al.*, *Nucl. Acids Res.*, 1991, *19*, 3345); acyl chains (Akashi *et al.*, *Chem. Lett.*, 1988, 1093; Yashima *et al.*, *J. Chromatogr.*, 1992, *603*, 111); polyvinyl alcohol (Schott, *J. Chromatogr.*, 1975, *115*, 461); alkyl chains (Goss *et al.*, *J. Chromatogr.*, 1990, *508*, 279); alkylamine chains (Pon *et al.*, *BioTechniques*, 1988, *6*, 768); biotin-avidin or biotin-streptavidin linkages (Kasher *et al.*, *Mol. Cell. Biol.*, 1986, *6*, 3117; Chodosh *et al.*, *Mol. Cell. Biol.*, 1986, *6*, 4723; Fishell *et al.*, *Methods Enzymol.*, 1990, *184*, 328); and art-recognized equivalents of any of the preceding linkers.

In a preferred embodiment of the invention, an *n*-aminoalkyl chain is the linker. In a particularly preferred embodiment of the invention, in which oligonucleotide chains constitute both the spacer (3) and the oligonucleotide probe (4) of the sensor array, a preferred linker (2) is an *n*-aminohexyl chain [*i.e.*, $NH_2-(CH_2)_6$].

The second linker or spacer (3) is optional and may be selected from a variety of chemical linking groups or chains. Any chemical group or chain capable of forming a chemical group or chain capable of forming an ultimately nonreactive linkage between the first linker (2) and the oligonucleotide probe (4) of the sensor array may be employed. A suitable spacer has the preferred characteristic of non-reactivity with

compounds introduced during the various steps of oligonucleotide synthesis. It will be appreciated by those skilled in the art that the chemical composition of the linker (2) and the probe oligonucleotide (4) will influence the choice of the spacer. Typically suitable spacers include, but are not limited to, oligopeptides; oligonucleotides; alkyl chains; polyamines; polyethylene glycols; oligosaccharides; and art-recognized equivalents of any of the preceding spacers.

In one set of preferred embodiments of the invention, the spacer is an alkyl chain, most preferably a C_1-C_{20} alkyl chain. In another set of preferred embodiments of the invention, the spacer is an oligonucleotide chain, particularly an oligonucleotide chain that comprises one or more chemical modifications that render it resistant to chemical attack. In this set of preferred embodiments, an oligodeoxyribonucleotide chain is particularly preferred. In a particularly preferred embodiment of the invention, poly(dT)$_{5-30}$ acts as the spacer of the matrix of the invention. This preferred spacer has the following advantages. This spacer is composed of nucleotides and is thus closely related in chemical properties to the preferred sensor array, i.e., an oligonucleotide. This chemical relatedness provides the benefit of placing the sensor array in a context that is likely to be appropriate for nucleic acid hybridization duplexing. Although other polynucleotides [e.g., poly(dA), poly(dG), poly(dC), *etc.*] might be employed

for the spacer, the preferred poly(dT) spacer is more chemically stable.

It will be appreciated by those skilled in the art that the first linker (2) and the second linker or spacer (3) can be combined into one linking unit. Furthermore, the linker and spacers need not comprise distinct chemical groups or chains. For example, an appropriate oligopeptide or oligonucleotide chain could function as a combined linker and spacer of the matrix of the invention. Thus, suitable linker/spacers include, but are not limited, to the linker and spacers described above. Methods of determining an appropriate linker/spacer length (for, e.g., the purpose of providing the optimal degree and specificity of hybridization between the sensor array and the target oligonucleotide) are known in the art (see, for example, Day *et al.*, *Biochem. J.*, 1991, *278*, 735). In certain instances, carbonate groups are specifically excluded from the linker (2) or spacer (3) of the sensor array of the invention. The carbonate moiety is excluded in some instances because it is relatively unstable to reagents used in some oligonucleotide syntheses and to contaminants (mainly bases) that may be found in solvents utilized in some oligonucleotide synthesis.

The oligonucleotide probe (4) of a sensor array has a nucleobase sequence that hybridizes specifically yet reversibly to a unique deletion sequence oligonucleotide. A preferred oligonucleotide probe is one having a nucleobase sequence that is the reverse complement of at least a portion

of the nucleobase sequence of the target deletion sequence oligonucleotide. The term "a portion" is intended to encompass at least five contiguous nucleobases uniquely derived from a section of the target deletion sequence oligonucleotide's sequence. In another preferred embodiment, the oligonucleotide probe is one having a nucleobase sequence that is (a) the reverse complement of the nucleobase sequence of the target deletion sequence oligonucleotide and (b) the same length as that of the target oligonucleotide.

A sensor array comprising an oligonucleotide probe having a nucleobase sequence that is the reverse complement of the nucleobase sequence of a target deletion sequence oligonucleotide will hybridize with high affinity to its corresponding target oligonucleotide but not to, e.g., other deletion oligonucleotides having different sequences. By stating that an oligonucleotide probe of the sensor array has a sequence that is the "reverse complement" of that of the nucleotide sequence of its target oligonucleotide, the following features are intended. As is known in the art, a nucleic acid duplex is formed of two antiparallel strands, *i.e.*, strands that hybridize to each other in a "head-to-tail" fashion:

```
Strand 1:   5'  ---------------------------> 3'
                 |||||||||||||||||||||||||||
Strand 2:   3' <---------------------------  5'
```

Specific nucleobases in the interior of a nucleic acid duplex bind to specific partner nucleobases to form a "base pair" (indicated by a "|" in the above representation). Among the

naturally occurring nucleobases, guanine (G) binds to cytosine (C), and adenine (A) binds to thymine (T) or uracil (U). Thus, in the above diagram, Strand 2 will have a nucleotide sequence that is the reverse complement of Strand 1, *i.e.*, Strand 2 will have, in "reverse" (3' to 5') order, the partner ("complement") nucleobases to those of Strand 1.

The sequence of the oligonucleotide of the sensor array can have reverse complementarity to the target oligonucleotide through a variety of equivalents. In addition to the equivalency of U (RNA) and T (DNA) as partners for A, other naturally occurring nucleobase equivalents are known, including 5-methylcytosine (m5c), 5-hydroxymethylcytosine (HMC) (C equivalents) and 5-hydroxymethyluracil (U equivalent). Furthermore, synthetic nucleobases which retain partner specificity are known in the art and include, for example, 7-deazaguanine, which retains specificity for C and is thus a G equivalent. Thus, reverse complementarity will not be altered by any chemical modification to a nucleobase in the nucleotide sequence of the affinity oligonucleotide which does not alter its specificity for the partner nucleobase in the target oligonucleotide.

The number of different probe oligonucleotides present in a given composition depends on the intended use for the composition and the nature of the sequence of the full-length "parent" oligonucleotide. For example, for the characterization of (*n*-1)-mers present in a synthetic

preparation of a "parent" oligonucleotide of length n, the number of different probe oligonucleotides is equal to

$$n - s,$$

wherein s is the number of (n-1) deletion sequence oligonucleotides that have the same sequence. For example, for the parent sequence

5'-G-G-C-T-T-T-T-C-3'
1 2 3 4 5 6 7 8

the deletion of a base at positions 4, 5, 6 and 7, or at positions 1 and 2, results in (n-1)-mers having identical sequences. As a result, there are 4 different possible oligonucleotide sequences resulting from the deletion of a single base from the parent sequence:

Parent: 5'-G-G-C-T-T-T-T-C-3'
Position: 1 2 3 4 5 6 7 8
(n-1)-mers:

G-C-T-T-T-T-C	(deletion at position 1 or 2)
G-G-T-T-T-T-C	(deletion at position 3)
G-G-C-T-T-T-C	(deletion at positions 4-7)
G-G-C-T-T-T-T	(deletion at position 8)

The nucleobase sequence of the oligonucleotide probe of the sensor array can be from 5 to about 50 nucleotides in length, preferably from 6 to about 25 nucleotides in length, more preferably from 8 to about 15 nucleotides in length. Although oligonucleotide probes of differing chemical

compositions (*e.g.*, oligodeoxynucleotides, oligoribonucleotides and peptide nucleic acids) can be employed in the invention, peptide nucleic acids and oligodeoxyribonucleotides are preferred in particular instances for the following reasons. Unlike RNA nucleases, for which no "universal" inhibitor is known, all characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents such as EDTA (Jarrett, *J. Chromatogr.*, 1993, *618*, 315); oligodeoxyribonucleotides can thus be more simply prevented from degradation than oligoribonucleotides. Peptide nucleic acids exhibit particularly stringent specificities for their complementary oligonucleotides, and may thus provide the best degree of separation from undesired derivative oligonucleotides in some instances.

In various embodiments of the invention, the oligonucleotide of the sensor array can incorporate one or more chemical modifications for the purpose of enhancing specific interactions with the target oligonucleotide. Such modifications may additionally or alternatively result in the oligonucleotide of the sensor array having increased resistance to degradative contaminants, *e.g.*, exonucleases. The target oligonucleotides may additionally or alternatively comprise such modifications, so long as reverse complementarity is maintained between the sequence of the target oligonucleotide and that of the probe oligonucleotide of the sensor array. Components of an oligonucleotide that

can be modified include the sugar (ribofuranosyl) portion, the nucleobase portion and one or more of the chemical linkages that make up an oligonucleotide's backbone. Specific chemical modifications of particular interest are described in the Examples.

It will be appreciated by those skilled in the art that the linker, spacer and probe oligonucleotide can be combined into one structurally linked unit. Furthermore, the linker, spacer and probe oligonucleotide need not comprise distinct chemical groups or chains. For example, an oligonucleotide of appropriate chain length and sequence could function as the linker, spacer and probe oligonucleotide of a sensor array.

It will be further appreciated by those skilled in the art that the spacer and probe oligonucleotide of a sensor array can be combined into one unit. Furthermore, the probe oligonucleotide and spacer need not comprise distinct chemical groups or chains. Thus, in a preferred embodiment, an aminohexyl group is the linker to the solid support, as it is easily attached to the 5' end of a oligonucleotide by a solid phase synthesizer. In this embodiment, an example of which is described in more detail in Examples 1 to 3, the probe oligonucleotide of a sensor array extends beyond its probe sequence (i.e., the sequence having reverse complementarity to all or a portion of a target deletion sequence oligonucleotide) to include a further nucleotide sequence which functions as the spacer of the sensor array.

Sensor arrays may be attached to the solid support by chemical conjugation of pre-synthesized sensor arrays to the

support. In an alternative embodiment, the sensor array is synthesized directly on the solid support (*i.e.*, *in situ*) rather than being separately synthesized and subsequently attached to the solid support. This embodiment is particularly useful when the components of the sensor array, and the components linking it to the solid support, are stable under the various conditions of synthesis and subsequent chemical steps (deprotection, deblocking and the like) necessary to prepare the matrix for use in the method of the invention. Examples of *in situ* synthesis of oligonucleotides on solid supports are known in the art (see, e.g., U.S. Patents Nos. 5,436,327 to Southern *et al.* and 5,429,807 to Matson *et al.*; Matson *et al.*, *Anal. Biochem.*, 1994, *217*, 306; Maskos *et al.*, *Nucl. Acids Res.*, 1992, *20*, 1679; Southern *et al.*, *Genomics*, 1992, *13*, 1008; Cashion *et al.*, *Nucl. Acids Res.*, 1977, *4*, 2593; Duncan *et al.*, *Anal. Biochem.*, 1988, *169*, 104).

Other features and advantages of the invention will be apparent to those skilled in the art upon reading and comprehending the disclosure. Those skilled in the art will recognize, or be able to ascertain through routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of the present invention.

EXAMPLES

The following examples illustrate the invention and are not intended to limit the same.

Example 1: Design of Probe Oligonucleotides

The number and sequences of different probe oligonucleotides present in a given composition depends on the intended use for the composition and the sequence of the parent oligonucleotide from which target deletion sequence oligonucleotides are derived. A synthetic oligonucleotide known as ISIS 2922 was chosen as an exemplary parent oligonucleotide for a series of experiments involving the methods and compositions of the invention.

ISIS 2922 is a synthetic 21 base (n-mer) antisense oligonucleotide targeted to cytomegalovirus having the following sequence (see SEQ ID NO:22 in U.S. Patent No. 5,442,049):

5'-GCGTTTGCTCTTCTTCTTGCG-3' SEQ ID NO:1.

in Table 1 wherein "^" indicates the position(s) of the deleted base in the particular (n-1) species. Because of the presence in ISIS 2922 of several stretches of two or more adjacent and identical residues, there are fewer (n-1) oligonucleotide sequences in this example than are possible for a 21-mer devoid of any such contiguous and identical

residues.

TABLE 1: (*n*-1) DELETION (^) DERIVATIVES OF ISIS 2922

Target No.	SEQ ID NO:	(*n*-1) Oligonucleotide Sequence
D1	2	5'-GCGTTTGCTCTTCTTCTTG^G-3'
D2	3	5'-GCGTTTGCTCTTCTTCTT^CG-3'
D3	4	5'-GCGTTTGCTCTTCTTCT^GCG-3'
D3	4	5'-GCGTTTGCTCTTCTTC^TGCG-3'
D4	5	5'-GCGTTTGCTCTTCTT^TTGCG-3'
D5	6	5'-GCGTTTGCTCTTCT^CTTGCG-3'
D5	6	5'-GCGTTTGCTCTTC^TCTTGCG-3'
D6	7	5'-GCGTTTGCTCTT^TTCTTGCG-3'
D7	8	5'-GCGTTTGCTCT^CTTCTTGCG-3'
D7	8	5'-GCGTTTGCTC^TCTTCTTGCG-3'
D8	9	5'-GCGTTTGCT^TTCTTCTTGCG-3'
D9	10	5'-GCGTTTGC^CTTCTTCTTGCG-3'
D10	11	5'-GCGTTTG^TCTTCTTCTTGCG-3'
D11	12	5'-GCGTTT^CTCTTCTTCTTGCG-3'
D12	13	5'-GCGTT^GCTCTTCTTCTTGCG-3'
D12	13	5'-GCGT^TGCTCTTCTTCTTGCG-3'
D12	13	5'-GCG^TTGCTCTTCTTCTTGCG-3'
D13	14	5'-GC^TTTGCTCTTCTTCTTGCG-3'
D14	15	5'-G^GTTTGCTCTTCTTCTTGCG-3'

For the sequence of ISIS 2922, there are fourteen different possible internal (n-1) deletion sequences, as shown

In order to specifically determine the presence and amount of each of these (n-1) impurities in a mixture, fourteen different oligonucleotide probes were designed, each of which is complementary to one of the above sequences. Each oligonucleotide probe only hybridizes with the corresponding target oligonucleotide of (n-1) deletion sequence through Watson-Crick double helix structure. The probes are natural or derivative oligonucleotides having a length of 20 bases.

Each of the probes used in the Examples of the present disclosure includes a 3'-terminal eight base sequence that is the reverse complement of the most 5' eight bases of a specific target (n-1) oligonucleotide (Table 2).

TABLE 2: NUCLEOTIDE SEQUENCES OF PROBES FOR (n-1) DELETION (^) DERIVATIVES OF ISIS 2922

Target No.:	Probe No:	SEQ ID NO:	Probe Oligonucleotide Sequence (3' => 5')
D1	P1	16	3'-AGAAGAAGAAC^C-$T_{15}(CH_2)_6NH_2$-5'
D2	P2	17	3'-AGAAGAAGAA^GC-$T_{15}(CH_2)_6NH_2$-5'
D3	P3	18	3'-AGAAGAAGA^CGC-$T_{15}(CH_2)_6NH_2$-5'
D4	P4	19	3'-AGAAGAA^AACGC-$T_{15}(CH_2)_6NH_2$-5'
D5	P5	20	3'-GAGAAGA^GAACG-$T_{15}(CH_2)_6NH_2$-5'
D6	P6	21	3'-CGAGAA^AAGAAC-$T_{15}(CH_2)_6NH_2$-5'
D7	P7	22	3'-AACGAGA^GAAGA-$T_{15}(CH_2)_6NH_2$-5'
D8	P8	23	3'-AAACGA^AAGAAG-$T_{15}(CH_2)_6NH_2$-5'
D9	P9	24	3'-CAAACG^GAAGAA-$T_{15}(CH_2)_6NH_2$-5'
D10	P10	25	3'-GCAAAC^AGAAGA-$T_{15}(CH_2)_6NH_2$-5'
D11	P11	26	3'-CGCAAA^GAGAAG-$T_{15}(CH_2)_6NH_2$-5'
D12	P12	27	3'-CGCAA^CGAGAAG-$T_{15}(CH_2)_6NH_2$-5'
D13	P13	28	3'-CG^AAACGAGAAG-$T_{15}(CH_2)_6NH_2$-5'
D14	P14	29	3'-C^CAAACGAGAAG-$T_{15}(CH_2)_6NH_2$-5'

As an example of the specificity of the above probes, the examplar Probe $P9^{EX}$, having the structure of

```
3'-AACGGAAGTTTTTTTTTTTTTT(CH2)6NH2-5'   SEQ ID NO:30
```

will be complementary to (match) the target oligonucleotide D9 (SEQ ID NO:10) over a length of eight (8) bases ("|" represents match and "*" stands for a mismatch between the strands):

```
5'-GCGTTTGCCTTCTTCTTGCG-3'                    D9    (SEQ ID NO:10)
       ||||||||
     3'-AACGGAAGTTTTTTTTTTTTTT(CH2)6NH2-5'          (SEQ ID NO:30)
```

and will have 1 to 3 bases of mismatch to all the other oligonucleotides of the (n-1) deletion sequences:

```
5'-GCGTTTGCTCTTCTTCTTGG-3'                    D1    (SEQ ID NO:2)
       ||||**|*
     3'-AACGGAAGTTTTTTTTTTTTTT(CH2)6NH2-5'          (SEQ ID NO:30)

5'-GCGTTTGCTCTTCTTCTTCG-3'                    D2    (SEQ ID NO:3)
       ||||**|*
     3'-AACGGAAGTTTTTTTTTTTTTT(CH2)6NH2-5'          (SEQ ID NO:30)

5'-GCGTTTGCTCTTCTTCTGCG-3'                    D3    (SEQ ID NO:4)
       ||||**|*
     3'-AACGGAAGTTTTTTTTTTTTTT(CH2)6NH2-5'          (SEQ ID NO:30)

5'-GCGTTTGCTCTTCTTTTGCG-3'                    D4    (SEQ ID NO:5)
       ||||**|*
     3'-AACGGAAGTTTTTTTTTTTTTT(CH2)6NH2-5'          (SEQ ID NO:30)

5'-GCGTTTGCTCTTCTCTTGCG-3'                    D5    (SEQ ID NO:6)
       ||||**|*
     3'-AACGGAAGTTTTTTTTTTTTTT(CH2)6NH2-5'          (SEQ ID NO:30)
```

```
5'-GCGTTTGCTCTTTTCTTGCG-3'                  D6    (SEQ ID NO:7)
        ||||**|*
     3'-AACGGAAGTTTTTTTTTTTTTT(CH2)6NH2-5'        (SEQ ID NO:30)

5'-GCGTTTGCTCTCTTCTTGCG-3'                  D7    (SEQ ID NO:8)
        ||||**||
     3'-AACGGAAGTTTTTTTTTTTTTT(CH2)6NH2-5'        (SEQ ID NO:30)

5'-GCGTTTGCTTTCTTCTTGCG-3'                  D8    (SEQ ID NO:9)
        ||||*|||
     3'-AACGGAAGTTTTTTTTTTTTTT(CH2)6NH2-5'        (SEQ ID NO:30)

5'-GCGTTTGTCTTCTTCTTGCG-3'                  D10   (SEQ ID NO:11)
        |||*||||
     3'-AACGGAAGTTTTTTTTTTTTTT(CH2)6NH2-5'        (SEQ ID NO:30)

5'-GCGTTTCTCTTCTTCTTGCG-3'                  D11   (SEQ ID NO:12)
        ||**||||
     3'-AACGGAAGTTTTTTTTTTTTTT(CH2)6NH2-5'        (SEQ ID NO:30)

5'-GCGTTGCTCTTCTTCTTGCG-3'                  D12   (SEQ ID NO:13)
        |***||||
     3'-AACGGAAGTTTTTTTTTTTTTT(CH2)6NH2-5'        (SEQ ID NO:30)

5'-GCTTTGCTCTTCTTCTTGCG-3'                  D13   (SEQ ID NO:14)
        |***||||
     3'-AACGGAAGTTTTTTTTTTTTTT(CH2)6NH2-5'        (SEQ ID NO:30)
```

and

```
5'-GGTTTGCTCTTCTTCTTGCG-3'                  D14   (SEQ ID NO:15)
        |***||||
     3'-AACGGAAGTTTTTTTTTTTTTT(CH2)6NH2-5'        (SEQ ID NO:30).
```

Similarly, the probe P9

3'-CAAACGGAAGAATTTTTTTTTTTTTTT$(CH_2)_6NH_2$-5' SEQ ID NO:24

will be complementary to (match) the target oligonucleotide D9

(SEQ ID NO:10) over a length of twelve (12) bases:

```
5'-GCGTTTGCCTTCTTCTTGCG-3'                D9   (SEQ ID NO:10)
     ||||||||||||
  3'-CAAACGGAAGAATTTTTTTTTTTTTT(CH2)6NH2-5'   (SEQ ID NO:24)
```

but will have mismatches of 1 to 4 base pairs to all the other (n-1) sequences:

```
5'-GCGTTTGCTCTTCTTCTTGG-3'                D1   (SEQ ID NO:2)
     ||||||**|**|
  3'-CAAACGGAAGAATTTTTTTTTTTTTT(CH2)6NH2-5'   (SEQ ID NO:24)

5'-GCGTTTGCTCTTCTTCTTCG-3'                D2   (SEQ ID NO:3)
     ||||||**|**|
  3'-CAAACGGAAGAATTTTTTTTTTTTTT(CH2)6NH2-5'   (SEQ ID NO:24)

5'-GCGTTTGCTCTTCTTCTGCG-3'                D3   (SEQ ID NO:4)
     ||||||**|**|
  3'-CAAACGGAAGAATTTTTTTTTTTTTT(CH2)6NH2-5'   (SEQ ID NO:24)

5'-GCGTTTGCTCTTCTTTTGCG-3'                D4   (SEQ ID NO:5)
     ||||||**|**|
  3'-CAAACGGAAGAATTTTTTTTTTTTTT(CH2)6NH2-5'   (SEQ ID NO:24)

5'-GCGTTTGCTCTTCTCTTGCG-3'                D5   (SEQ ID NO:6)
     ||||||**|**|
  3'-CAAACGGAAGAATTTTTTTTTTTTTT(CH2)6NH2-5'   (SEQ ID NO:24)

5'-GCGTTTGCTCTTTTCTTGCG-3'                D6   (SEQ ID NO:7)
     ||||||**|*||
  3'-CAAACGGAAGAATTTTTTTTTTTTTT(CH2)6NH2-5'   (SEQ ID NO:24)

5'-GCGTTTGCTCTCTTCTTGCG-3'                D7   (SEQ ID NO:8)
     ||||||**||||
  3'-CAAACGGAAGAATTTTTTTTTTTTTT(CH2)6NH2-5'   (SEQ ID NO:24)

5'-GCGTTTGCTTTCTTCTTGCG-3'                D8   (SEQ ID NO:9)
     ||||||*|||||
  3'-CAAACGGAAGAATTTTTTTTTTTTTT(CH2)6NH2-5'   (SEQ ID NO:24)
```

```
5'-GCGTTTGTCTTCTTCTTGCG-3'                   D10   (SEQ ID NO:11)
      |||||*||||||
  3'-CAAACGGAAGAATTTTTTTTTTTTTT(CH2)6NH2-5'  (SEQ ID NO:24)

5'-GCGTTTCTCTTCTTCTTGCG-3'                   D11   (SEQ ID NO:12)
      ||||**||||||
  3'-CAAACGGAAGAATTTTTTTTTTTTTT(CH2)6NH2-5'  (SEQ ID NO:24)

5'-GCGTTGCTCTTCTTCTTGCG-3'                   D12   (SEQ ID NO:13)
      |||***||||||
  3'-CAAACGGAAGAATTTTTTTTTTTTTT(CH2)6NH2-5'  (SEQ ID NO:24)

5'-GCTTTGCTCTTCTTCTTGCG-3'                   D13   (SEQ ID NO:14)
      *||***||||||
  3'-CAAACGGAAGAATTTTTTTTTTTTTT(CH2)6NH2-5'  (SEQ ID NO:24),
```

and

```
5'-GGTTTGCTCTTCTTCTTGCG-3'                   D14   (SEQ ID NO:15)
      *||***||||||
  3'-CAAACGGAAGAATTTTTTTTTTTTTT(CH2)6NH2-5'  (SEQ ID NO:24).
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Example 2: Synthesis of Oligonucleotides

A. General Synthetic Techniques: Oligonucleotides were synthesized on an automated DNA synthesizer using standard phosphoramidite chemistry with oxidation using iodine. Beta-cyanoethyldiisopropyl phosphoramidites were purchased from Applied Biosystems (Foster City, CA). For phosphorothioate oligonucleotides, the standard oxidation bottle was replaced by a 0.2 M solution of 3*H*-1,2-benzodithiole-3-one-1,1-dioxide in acetonitrile for the stepwise thiation of the phosphite linkages.

The synthesis of 2'-O-methyl- (a.k.a. 2'-methoxy-) phosphorothioate oligonucleotides is according to the procedures set forth above substituting 2'-O-methyl β-cyanoethyldiisopropyl phosphoramidites (Chemgenes, Needham, MA) for standard phosphoramidites and increasing the wait cycle after the pulse delivery of tetrazole and base to 360

seconds.

Similarly, 2'-O-propyl- (a.k.a 2'-propoxy-) phosphoro thioate oligonucleotides are prepared by slight modifications of this procedure and essentially according to procedures disclosed in U.S. patent application Serial No. 08/383,666, filed February 3, 1995, which is assigned to the same assignee as the instant application and which is incorporated by reference herein.

The 2'-fluoro-phosphorothioate oligonucleotides of the invention are synthesized using 5'-dimethoxytrityl-3'-phosphoramidites and prepared as disclosed in U.S. patent application Serial No. 08/383,666, filed February 3, 1995, and U.S. Patent 5,459,255, which issued October 8, 1996, both of which are assigned to the same assignee as the instant application and which are incorporated by reference herein. The 2'-fluoro-oligonucleotides are prepared using phosphoramidite chemistry and a slight modification of the standard DNA synthesis protocol (*i.e.*, deprotection was effected using methanolic ammonia at room temperature).

PNA antisense analogs are prepared essentially as described in U.S. Patents Nos. 5,539,082 and 5,539,083, both of which (1) issued July 23, 1996, (2) are assigned to the same assignee as the instant application and (3) are incorporated by reference herein.

Oligonucleotides comprising 2,6-diaminopurine are prepared using compounds described in U.S. Patent No. 5,506,351 which issued April 9, 1996, and which is assigned to the same assignee as the instant application and incorporated by reference herein, and materials and methods described by Gaffney *et al.* (*Tetrahedron*, 1984, *40*:3), Chollet *et al.*, (*Nucl. Acids Res.*, 1988, *16*:305) and Prosnyak *et al.* (*Genomics*, 1994, *21*:490). Oligonucleotides comprising 2,6-diaminopurine can also be prepared by enzymatic means (Bailly *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 1996, *93*:13623).

The 2'-methoxyethoxy oligonucleotides of the invention were synthesized essentially according to the methods of

Martin *et al.* (*Helv. Chim. Acta*, 1995, *78*, 486). For ease of synthesis, the 3' nucleotide of the 2'-methoxyethoxy oligonucleotides was a deoxynucleotide, and 2'-O-$CH_2CH_2OCH_3$-cytosines were 5-methyl cytosines, which were synthesized according to the procedures described below.

B. Synthesis of 5-Methyl Cytosine Monomers:

2,2'-Anhydro[1-(β-D-arabinofuranosyl)-5-methyluridine]: 5-Methyluridine (ribosylthymine, commercially available through Yamasa, Choshi, Japan) (72.0 g, 0.279 M), diphenylcarbonate (90.0 g, 0.420 M) and sodium bicarbonate (2.0 g, 0.024 M) were added to *N,N*-dimethylformamide (DMF, 300 mL). The mixture was heated to reflux, with stirring, allowing the evolved carbon dioxide gas to be released in a controlled manner. After 1 hour, the slightly darkened solution was concentrated under reduced pressure. The resulting syrup was poured into diethylether (2.5 L), with stirring. The product formed a gum. The ether was decanted and the residue was dissolved in a minimum amount of methanol (ca. 400 mL). The solution was poured into fresh ether (2.5 L) to yield a stiff gum. The ether was decanted and the gum was dried in a vacuum oven (60 C at 1 mm Hg for 24 h) to give a solid which was crushed to a light tan powder (57 g, 85% crude yield). The material was used as is for further reactions.

2'-O-Methoxyethyl-5-methyluridine: 2,2'-Anhydro-5-methyluridine (195 g, 0.81 M), tris(2-methoxyethyl)borate (231 g, 0.98 M) and 2-methoxyethanol (1.2 L) were added to a 2 L stainless steel pressure vessel and placed in a pre-heated oil bath at 160 C. After heating for 48 hours at 155-160 C, the vessel was opened and the solution evaporated to dryness and triturated with methanol (200 mL). The residue was suspended in hot acetone (1 L). The insoluble salts were filtered, washed with acetone (150 mL) and the filtrate evaporated. The residue (280 g) was dissolved in CH_3CN (600 mL) and

evaporated. A silica gel column (3 kg) was packed in CH_2Cl_2/acetone/methanol (20:5:3) containing 0.5% Et_3NH. The residue was dissolved in CH_2Cl_2 (250 mL) and adsorbed onto silica (150 g) prior to loading onto the column. The product was eluted with the packing solvent to give 160 g (63%) of product.

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine: 2'-O-Methoxyethyl-5-methyluridine (160 g, 0.506 M) was co-evaporated with pyridine (250 mL) and the dried residue dissolved in pyridine (1.3 L). A first aliquot of dimethoxytrityl chloride (94.3 g, 0.278 M) was added and the mixture stirred at room temperature for one hour. A second aliquot of dimethoxytrityl chloride (94.3 g, 0.278 M) was added and the reaction stirred for an additional one hour. Methanol (170 mL) was then added to stop the reaction. High pressure liquid chromatography (HPLC) showed the presence of approximately 70% product. The solvent was evaporated and triturated with CH_3CN (200 mL). The residue was dissolved in $CHCl_3$ (1.5 L) and extracted with 2x 500 mL of saturated $NaHCO_3$ and 2x 500 mL of saturated NaCl. The organic phase was dried over Na_2SO_4, filtered and evaporated. 275 g of residue was obtained. The residue was purified on a 3.5 kg silica gel column, packed and eluted with EtOAc/Hexane/Acetone (5:5:1) containing 0.5% Et_3NH. The pure fractions were evaporated to give 164 g of product. Approximately 20 g additional was obtained from the impure fractions to give a total yield of 183 g (57%).

3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine: 2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (106 g, 0.167 M), DMF/pyridine (750 mL of a 3:1 mixture prepared from 562 mL of DMF and 188 mL of pyridine) and acetic anhydride (24.38 mL, 0.258 M) were combined and stirred at room temperature for 24 hours. The reaction was monitored by thin layer chromatography (tlc) by first quenching the tlc sample with the addition of MeOH. Upon completion of the reaction, as judged by tlc, MeOH (50 mL) was

added and the mixture evaporated at 35 C. The residue was dissolved in $CHCl_3$ (800 mL) and extracted with 2x 200 mL of saturated sodium bicarbonate and 2x 200 mL of saturated NaCl. The water layers were back extracted with 200 mL of $CHCl_3$. The combined organics were dried with sodium sulfate and evaporated to give 122 g of residue (approximately 90% product). The residue was purified on a 3.5 kg silica gel column and eluted using EtOAc/Hexane (4:1). Pure product fractions were evaporated to yield 96 g (84%).

3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine: A first solution was prepared by dissolving 3'-O-acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (96 g, 0.144 M) in CH_3CN (700 mL) and set aside. Triethylamine (189 mL, 1.44 M) was added to a solution of triazole (90 g, 1.3 M) in CH_3CN (1 L), cooled to -5 C and stirred for 0.5 h using an overhead stirrer. $POCl_3$ was added dropwise, over a 30 minute period, to the stirred solution maintained at 0-10 C, and the resulting mixture stirred for an additional 2 hours. The first solution was added dropwise, over a 45 minute period, to the later solution. The resulting reaction mixture was stored overnight in a cold room. Salts were filtered from the reaction mixture and the solution was evaporated. The residue was dissolved in EtOAc (1 L) and the insoluble solids were removed by filtration. The filtrate was washed with 1x 300 mL of $NaHCO_3$ and 2x 300 mL of saturated NaCl, dried over sodium sulfate and evaporated. The residue was triturated with EtOAc to give the title compound.

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine: A solution of 3'-O-acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine (103 g, 0.141 M) in dioxane (500 mL) and NH_4OH (30 mL) was stirred at room temperature for 2 hours. The dioxane solution was evaporated and the residue azeotroped with MeOH (2x 200 mL). The residue was dissolved in MeOH (300 mL) and transferred to a 2 liter stainless steel pressure vessel. Methanol (400 mL) saturated with NH_3 gas was added and the vessel heated to 100 C for 2

hours (thin layer chromatography, tlc, showed complete conversion). The vessel contents were evaporated to dryness and the residue was dissolved in EtOAc (500 mL) and washed once with saturated NaCl (200 mL). The organics were dried over sodium sulfate and the solvent was evaporated to give 85 g (95%) of the title compound.

N^4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine: 2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine (85 g, 0.134 M) was dissolved in DMF (800 mL) and benzoic anhydride (37.2 g, 0.165 M) was added with stirring. After stirring for 3 hours, tlc showed the reaction to be approximately 95% complete. The solvent was evaporated and the residue azeotroped with MeOH (200 mL). The residue was dissolved in $CHCl_3$ (700 mL) and extracted with saturated $NaHCO_3$ (2x 300 mL) and saturated NaCl (2x 300 mL), dried over $MgSO_4$ and evaporated to give a residue (96 g). The residue was chromatographed on a 1.5 kg silica column using EtOAc/Hexane (1:1) containing 0.5% Et_3NH as the eluting solvent. The pure product fractions were evaporated to give 90 g (90%) of the title compound.

N^4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine-3'-amidite: N^4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine (74 g, 0.10 M) was dissolved in CH_2Cl_2 (1 L). Tetrazole diisopropylamine (7.1 g) and 2-cyanoethoxy-tetra(isopropyl)phosphite (40.5 mL, 0.123 M) were added with stirring, under a nitrogen atmosphere. The resulting mixture was stirred for 20 hours at room temperature (tlc showed the reaction to be 95% complete). The reaction mixture was extracted with saturated $NaHCO_3$ (1x 300 mL) and saturated NaCl (3x 300 mL). The aqueous washes were back-extracted with CH_2Cl_2 (300 mL), and the extracts were combined, dried over $MgSO_4$ and concentrated. The residue obtained was chromatographed on a 1.5 kg silica column using EtOAc\Hexane (3:1) as the eluting solvent. The pure fractions were combined to give 90.6 g (87%) of the title compound.

C. 2'-O-(2-Methoxyethyl) Modified Amidites

2'-O-Methoxyethyl-substituted nucleoside amidites are prepared as follows, or alternatively, as per the methods of Martin (*Helvetica Chimica Acta*, 1995, *78*, 486).

2,2'-Anhydro[1-(beta-D-arabinofuranosyl)-5-methyluridine]: 5-Methyluridine (ribosylthymine, commercially available through Yamasa, Choshi, Japan) (72.0 g, 0.279 M), diphenylcarbonate (90.0 g, 0.420 M) and sodium bicarbonate (2.0 g, 0.024 M) were added to DMF (300 mL). The mixture was heated to reflux, with stirring, allowing the evolved carbon dioxide gas to be released in a controlled manner. After 1 hour, the slightly darkened solution was concentrated under reduced pressure. The resulting syrup was poured into diethylether (2.5 L), with stirring. The product formed a gum. The ether was decanted and the residue was dissolved in a minimum amount of methanol (ca. 400 mL). The solution was poured into fresh ether (2.5 L) to yield a stiff gum. The ether was decanted and the gum was dried in a vacuum oven (60°C at 1 mm Hg for 24 h) to give a solid that was crushed to a light tan powder (57 g, 85% crude yield). The NMR spectrum was consistent with the structure, contaminated with phenol as its sodium salt (ca. 5%). The material was used as is for further reactions (or it can be purified further by column chromatography using a gradient of methanol in ethyl acetate (10-25%) to give a white solid, mp 222-4°C).

2'-O-Methoxyethyl-5-methyluridine: 2,2'-Anhydro-5-methyluridine (195 g, 0.81 M), tris(2-methoxyethyl)borate (231 g, 0.98 M) and 2-methoxyethanol (1.2 L) were added to a 2 L stainless steel pressure vessel and placed in a pre-heated oil bath at 160°C. After heating for 48 hours at 155-160°C, the vessel was opened and the solution evaporated to dryness and triturated with MeOH (200 mL). The residue was suspended in hot acetone (1 L). The insoluble salts were filtered, washed with acetone (150 mL) and the filtrate evaporated. The residue (280 g) was dissolved in CH_3CN (600 mL) and evaporated. A silica gel column (3 kg) was packed in CH_2Cl_2/acetone/MeOH (20:5:3) containing 0.5% Et_3NH. The

residue was dissolved in CH_2Cl_2 (250 mL) and adsorbed onto silica (150 g) prior to loading onto the column. The product was eluted with the packing solvent to give 160 g (63%) of product. Additional material was obtained by reworking impure fractions.

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine: 2'-O-Methoxyethyl-5-methyluridine (160 g, 0.506 M) was co-evaporated with pyridine (250 mL) and the dried residue dissolved in pyridine (1.3 L). A first aliquot of dimethoxytrityl chloride (94.3 g, 0.278 M) was added and the mixture stirred at room temperature for one hour. A second aliquot of dimethoxytrityl chloride (94.3 g, 0.278 M) was added and the reaction stirred for an additional one hour. Methanol (170 mL) was then added to stop the reaction. HPLC showed the presence of approximately 70% product. The solvent was evaporated and triturated with CH_3CN (200 mL). The residue was dissolved in $CHCl_3$ (1.5 L) and extracted with 2x 500 mL of saturated $NaHCO_3$ and 2x500 mL of saturated NaCl. The organic phase was dried over Na_2SO_4, filtered and evaporated. 275 g of residue was obtained. The residue was purified on a 3.5 kg silica gel column, packed and eluted with EtOAc/Hexane/Acetone (5:5:1) containing 0.5% Et_3NH. The pure fractions were evaporated to give 164 g of product. Approximately 20 g additional was obtained from the impure fractions to give a total yield of 183 g (57%).

3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine: 2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (106 g, 0.167 M), DMF/pyridine (750 mL of a 3:1 mixture prepared from 562 mL of DMF and 188 mL of pyridine) and acetic anhydride (24.38 mL, 0.258 M) were combined and stirred at room temperature for 24 hours. The reaction was monitored by tlc by first quenching the tlc sample with the addition of MeOH. Upon completion of the reaction, as judged by tlc, MeOH (50 mL) was added and the mixture evaporated at 35°C. The residue was dissolved in $CHCl_3$ (800 mL) and extracted with 2x 200 mL of saturated sodium bicarbonate and

2x 200 mL of saturated NaCl. The water layers were back extracted with 200 mL of $CHCl_3$. The combined organics were dried with sodium sulfate and evaporated to give 122 g of residue (approx. 90% product). The residue was purified on a 3.5 kg silica gel column and eluted using EtOAc/Hexane(4:1). Pure product fractions were evaporated to yield 96 g (84%). An additional 1.5 g was recovered from later fractions.

3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine: A first solution was prepared by dissolving 3'-O-acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (96 g, 0.144 M) in CH_3CN (700 mL) and set aside. Triethylamine (189 mL, 1.44 M) was added to a solution of triazole (90 g, 1.3 M) in CH_3CN (1 L), cooled to -5°C and stirred for 0.5 h using an overhead stirrer. $POCl_3$ was added dropwise, over a 30 minute period, to the stirred solution maintained at 0-10°C, and the resulting mixture stirred for an additional 2 hours. The first solution was added dropwise, over a 45 minute period, to the later solution. The resulting reaction mixture was stored overnight in a cold room. Salts were filtered from the reaction mixture and the solution was evaporated. The residue was dissolved in EtOAc (1 L) and the insoluble solids were removed by filtration. The filtrate was washed once with 300 mL of $NaHCO_3$ and 2x 300 mL of saturated NaCl, dried over sodium sulfate and evaporated. The residue was triturated with EtOAc to give the title compound.

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyl cytidine: A solution of 3'-O-acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine (103 g, 0.141 M) in dioxane (500 mL) and NH_4OH (30 mL) was stirred at room temperature for 2 hours. The dioxane solution was evaporated and the residue azeotroped with MeOH (2x 200 mL). The residue was dissolved in MeOH (300 mL) and transferred to a 2 liter stainless steel pressure vessel. MeOH (400 mL) saturated with NH_3 gas was added and the vessel heated to 100°C for 2 hours (tlc showed complete conversion). The vessel contents were evaporated to dryness and the residue was dissolved in EtOAc

(500 mL) and washed once with saturated NaCl (200 mL). The organics were dried over sodium sulfate and the solvent was evaporated to give 85 g (95%) of the title compound.

N^4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine: 2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine (85 g, 0.134 M) was dissolved in DMF (800 mL) and benzoic anhydride (37.2 g, 0.165 M) was added with stirring. After stirring for 3 hours, tlc showed the reaction to be approximately 95% complete. The solvent was evaporated and the residue azeotroped with MeOH (200 mL). The residue was dissolved in $CHCl_3$ (700 mL) and extracted with saturated $NaHCO_3$ (2x 300 mL) and saturated NaCl (2x300 mL), dried over $MgSO_4$ and evaporated to give a residue (96 g). The residue was chromatographed on a 1.5 kg silica column using EtOAc/Hexane (1:1) containing 0.5% Et_3NH as the eluting solvent. The pure product fractions were evaporated to give 90 g (90%) of the title compound.

N^4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine-3'-amidite: N^4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine (74 g, 0.10 M) was dissolved in CH_2Cl_2 (1 L). Tetrazole diisopropylamine (7.1 g) and 2-cyanoethoxy-tetra(isopropyl)phosphite (40.5 mL, 0.123 M) were added with stirring, under a nitrogen atmosphere. The resulting mixture was stirred for 20 hours at room temperature (tlc showed the reaction to be 95% complete). The reaction mixture was extracted with saturated $NaHCO_3$ (1x 300 mL) and saturated NaCl (3x 300 mL). The aqueous washes were back-extracted with CH_2Cl_2 (300 mL), and the extracts were combined, dried over $MgSO_4$ and concentrated. The residue obtained was chromatographed on a 1.5 kg silica column using EtOAc\Hexane (3:1) as the eluting solvent. The pure fractions were combined to give 90.6 g (87%) of the title compound.

2'-(Aminooxyethyl) nucleoside amidites and 2'-(dimethylaminooxyethyl) nucleoside amidites: Aminooxyethyl

and dimethylaminooxyethyl amidites are prepared as per the methods of United States patent applications serial number 10/037,143, filed February 14, 1998, and serial number 09/016,520, filed January 30, 1998, each of which is commonly owned with the instant application and is herein incorporated by reference.

D. Synthesis of Other Oligonucleotides

Unsubstituted and substituted phosphodiester (P=O) oligonucleotides are synthesized on an automated DNA synthesizer (Applied Biosystems model 380B) using standard phosphoramidite chemistry with oxidation by iodine.

Phosphorothioates (P=S) are synthesized as for the phosphodiester oligonucleotides except the standard oxidation bottle was replaced by 0.2 M solution of 3H-1,2-benzodithiole-3-one 1,1-dioxide in acetonitrile for the stepwise thiation of the phosphite linkages. The thiation wait step was increased to 68 sec and was followed by the capping step. After cleavage from the CPG column and deblocking in concentrated ammonium hydroxide at 55°C (18 hr), the oligonucleotides were purified by precipitating twice with 2.5 volumes of ethanol from a 0.5 M NaCl solution.

Phosphinate oligonucleotides are prepared as described in U.S. Patent 5,508,270, herein incorporated by reference.

Alkyl phosphonate oligonucleotides are prepared as described in U.S. Patent 4,469,863, herein incorporated by reference.

3'-Deoxy-3'-methylene phosphonate oligonucleotides are prepared as described in U.S. Patents 5,610,289 or 5,625,050, herein incorporated by reference.

Phosphoramidite oligonucleotides are prepared as described in U.S. Patent, 5,256,775 or U.S. Patent 5,366,878, herein incorporated by reference.

Alkylphosphonothioate oligonucleotides are prepared as described in published PCT applications PCT/US94/00902 and PCT/US93/06976 (published as WO 94/17093 and WO 94/02499,

respectively), herein incorporated by reference.

3'-Deoxy-3'-amino phosphoramidate oligonucleotides are prepared as described in U.S. Patent 5,476,925, herein incorporated by reference.

Phosphotriester oligonucleotides are prepared as described in U.S. Patent 5,023,243, herein incorporated by reference.

Borano phosphate oligonucleotides are prepared as described in U.S. Patents 5,130,302 and 5,177,198, both herein incorporated by reference.

Methylenemethylimino linked oligonucleosides, also identified as MMI linked oligonucleosides, methylenedimethylhydrazo linked oligonucleosides, also identified as MDH linked oligonucleosides, and methylenecarbonylamino linked oligonucleosides, also identified as amide-3 linked oligonucleosides, and methyleneaminocarbonyl linked oligonucleosides, also identified as amide-4 linked oligonucleosides, as well as mixed backbone compounds having, for instance, alternating MMI and P=O or P=S linkages are prepared as described in U.S. Patents 5,378,825, 5,386,023, 5,489,677, 5,602,240 and 5,610,289, all of which are herein incorporated by reference.

Formacetal and thioformacetal linked oligonucleosides are prepared as described in U.S. Patents 5,264,562 and 5,264,564, herein incorporated by reference.

Ethylene oxide linked oligonucleosides are prepared as described in U.S. Patent 5,223,618, herein incorporated by reference.

E. Oligonucleotide Purification: After cleavage from the controlled pore glass (CPG) column (Applied Biosystems) and deblocking in concentrated ammonium hydroxide, at 55°C for 18 hours, the oligonucleotides were purified by precipitation 2x from 0.5 M NaCl with 2.5 volumes of ethanol followed by further purification by reverse phase high liquid pressure chromatography (HPLC). Analytical gel electrophoresis was accomplished in 20% acrylamide, 8 M urea and 45 mM Tris-borate

buffer (pH 7).

Example 3: Coupling the Solid Support (1) to a Linker (2)

Glass material is preferred as a solid support for the probes. Commercial glass is a rigid liquid product of fusion with inorganic ingredients. Silica (SiO2) derived from sand provides the structure backbone. There are Si-OH bonds on the glass surface. Both microscopic glass slides and derivatized controlled-pore glass can be used as the solid support to attach the modified oligonucleotide probe.

The glass surface is modified to form an amino-reactive terminal. For the microscopic glass slides, the modification can be achieved by treating the glass surface (GS) with a chemical, $(CH_3O)_3Si(CH_2)_3NCS$:

$$\begin{array}{l}\text{Glass surface-SiOH} \\ \qquad + \qquad \text{------->}\ (GS)\text{-Si-O-Si}(CH_3O)_2(CH_2)_3NCS \\ (CH_3O)_3Si(CH_2)_3NCS\end{array}$$

The synthesis of $(CH_3O)_3Si(CH_2)_3NCS$ is known in the art, *e.g.*, by the reaction of $(CH_3O)_3Si(CH_2)_3$ with a large excess of carbon disulfide to form dithiocarbamic acid followed by the conversion to corresponding isothiocyanates with cyanamide in tetrahydrofuran (THF) (Yamamoto *et al.*, *OPPI Briefs*, 1992, *24*, 346; Yamamoto *et al.*, *OPPI Briefs*, 1994, *26*, 555).

Another way to form the amino-active terminal on the glass surface is to use two step reactions. The first reaction forms a primary amine, and then in the second reaction, this primary amine reacts with 1,4-phenylene diisothiocyanate to form the amino reactive phenylisothiocyanate group.

$$\text{Glass surface-SiOH} + (CH_3O)_3Si(CH_2)_3NH_2$$

$$\text{----->}\ \text{Glass surface-Si-O-Si}(OCH_3)_2(CH_2)_3NH_2$$

and

Glass surface-Si-O-Si$(CH_3O)_2(CH_2)_3NH_2$ + S=C=N-Ph-N=C=S

-----> Glass surface-Si-O-Si$(OCH_3)_2(CH_2)_3$NHC(=S)NH-Ph-N=C=S

As for the commercially available derivatized CPG, aminopropyl CPG or long chain amino CPG is preferred. In both cases, the primary amine is located on the glass surface and modification is achieved by reacting this amino group of the glass surface with 1,4-phenylene diisothiocyanate to form the amino reactive phenylisothiocyanate group:

CPG-$(CH_2)_3NH_2$ + S=C=N-Ph-N=C=S

-----> CPG-$(CH_2)_3$NHC(=S)NH-Ph-N=C=S

or

CPG-$(CH_2)_nNH_2$ + S=C=N-Ph-N=C=S

-----> CPG-$(CH_2)_n$NHC(=S)NH-Ph-N=C=S

Example 4: Coupling of a [Probe Oligonucleotide (4)/Spacer (3)] Unit to a Linker (2)

Under mildly alkaline condition, the primary amine group of each probe reacts with the isothiocyanate group in the glass surface to form a thiocarbamyl adduct. The probe (in the following example reactions, probe $P9^{EX}$, SEQ ID NO:30) is therefore covalently attached to the glass surface (**GS**):

(**GS**)-Si-O-Si$(CH_3O)_2(CH_2)_3$NCS + **AACGGAAGT**$_{15}(CH_2)_6NH_2$

-----> (GS)-Si-O-Si$(CH_3O)_2(CH_2)_3$NHCSNH$(CH_2)_6T_{15}$**GAAGGCAA**-5'

or

(GS)-Si-O-Si$(OCH_3)_2(CH_2)_3$NHC(=S)NH-Ph-N=C=S +

AACGGAAGT$_{15}(CH_2)_6NH_2$ ----------->

(GS)-Si-O-Si$(OCH_3)_2(CH_2)_3$NHCSNH-Ph-NHCSNH$(CH_2)_6T_{15}$**GAAGGCAA**

Example 5: Labeling of Oligonucleotides

A. Immobilization Efficiency: In order to detect the result of immobilization, the [probe oligonucleotide/spacer] unit of the sensor array are labeled with moieties which can produce detectable signals. Labeling can be achieved by attaching a fluorescent dye, or a radioisotope such as ^{32}P or ^{35}S.

1. Fluorescent Labeling: Fluorescent labeling can be achieved by reacting a commercially available nucleoside terminator labeled with a dye (for example, carboxyfluorescein (FAM)) such as, for example, ddC-5FAM, with the probe (in the following example reactions, probe P9^{EX}, SEQ ID NO:30) in the presence of an enzyme, deoxynucleotidyl transferase:

$NH_2(CH2)_6T_{15}$GAAGGCAA-3' + ddCTP-5FAM

$$\xrightarrow[\text{37°C, 1 hour}]{\text{Deoxynucleotidyl Transferase}}$$

$NH_2(CH_2)_6T_{15}$GAAGGCAAC-5FAM

2. Radioisotope Labeling: Radioisotope labeling can

be achieved by reacting [α-^{35}S]dATP, or [α-^{32}P]dATP with the probe (in the following example reactions, probe P9^{EX}, SEQ ID NO:30) in the presence of the same enzyme:

$NH_2(CH_2)_6T_{15}$GAAGGCAA-3' + [α-^{35}S]dATP

$$\xrightarrow[37^\circ C,\ 1\ hour]{\text{Deoxynucleotidyl Transferase}}$$

$NH2(CH_2)_6T_{15}$GAAGGCAA-^{35}S-A

or

$NH_2(CH_2)_6T_{15}$GAAGGCAA-3' + [α-^{32}P]dATP

$$\xrightarrow[37^\circ C,\ 1\ hour]{\text{Deoxynucleotidyl Transferase}}$$

$NH_2(CH_2)_6T_{15}$GAAGGCAA-^{32}P-A

3. **Enzymatic Labeling:** Oligonucleotides may also be labeled with enzymatic groups, or groups that bind an enzyme (e.g., biotin), and subsequently detected by chemical reactions catalyzed by such enzymes. A variety of enzyme-oligonucleotide conjugates, and means of preparing such conjugates, are known in the art (see, e.g., Ruth, Chapter 6 *In: Methods in Molecular Biology, Vol. 26: Protocols for Oligonucleotide Conjugates*, Agrawal, S., Ed., 1994, Humana Press Inc., Totowa, NJ, pages 167-185).

Although optimal immobilization conditions are established using labeled probe oligonucleotides or labeled [probe oligonucleotide/spacer] units, it is not desirable to label the probe oligonucleotides during the routine operation of the invention. Therefore, unlabeled oligonucleotide probes are immobilized under the optimal conditions to form the sensor array to which labeled target deletion sequence

oligonucleotides are applied for hybridization.

B. Labeling of Target (*n*-1) Oligonucleotides: The target oligonucleotides of (*n*-1) deletion sequences can be labeled with fluorescent dye or radioisotope according to the following example reactions. In the following example reactions, target oligonucleotide D11 (SEQ ID NO:12) is used as the target (*n*-1) oligonucleotide.

1. Fluorescent Labeling: Fluorescent labeling can be achieved by reacting a commercially available dye labeled nucleoside terminator, ddC-5FAM with the target oligonucleotide in the presence of deoxynucleotidyl transferase:

5'-GCGTTTCTCTTCTTCTTGCG-3' + ddCTP-5FAM

Deoxynucleotidyl Transferase
------------------------------------->
37°C, 1 hour

GCGTTTCTCTTCTTCTTGCGC-5FAM

2. Radioisotope Labeling: The target oligonucleotides of (*n*-1) deletion sequence oligonucleotides are labeled with radioisotope (such as, for example, ^{35}S or ^{32}P) at either the 3' end with deoxynucleotidyl transferase or the 5' end with T4 nucleotidyl kinase. For 3' end radiolabeling, either of the following reactions can, for example, be used:

5'-GCGTTTCTCTTCTTCTTGCG-3' + [α-^{35}S]dATP

Deoxynucleotidyl Transferase
------------------------------------->
37°C, 1 hour

GCGTTTCTCTTCTTCTTGCG-^{35}S-A

or

5'-GCGTTTCTCTTCTTCTTGCG-3' + [α-^{32}P] dATP

Deoxynucleotidyl Transferase

$\xrightarrow[37^\circ C,\ 1\ hour]{}$

GCGTTTCTCTTCTTCTTGCG-^{32}P-A

In similar fashion, for 5' end radiolabeling, either of the following reactions can, for example, be used:

5'-GCGTTTCTCTTCTTCTTGCG-3' + [γ-^{35}S] dATP

T4 Nucleotidyl Kinase

$\xrightarrow[37^\circ C,\ 1\ hour]{}$

^{35}S-GCGTTTCTCTTCTTCTTGCG

or

5'-GCGTTTCTCTTCTTCTTGCG-3' + [γ-^{32}P] dATP

T4 Nucleotidyl Kinase

$\xrightarrow[37^\circ C,\ 1\ hour]{}$

^{32}P-GCGTTTCTCTTCTTCTTGCG

C. Results: Using terminal transferase, the oligonucleotide probe P11 (SEQ ID NO:26) was radiolabeled at its 3' end with ^{35}αdATP. The labeled probe was attached to glass microscope slides according to the methods described in Example 4. For each sample, 2 uL of labeled probe, at a concentration of 0.5 pmol/uL in 10^{-3} N NaOH, was applied to the glass slides. The alkaline conditions led to immobilization of the labeled probe according to reactions detailed in Example 4. The glass slides were then rinsed with

water and allowed to dry, and then exposed to emulsion coated film for autoradiography. The resulting negatives were read on a densitometer (Molecular Dynamics, Sunnyvale, CA) to determine the relative intensities of each spot. The results (Table 3) demonstrate that immobilization occurs rapidly over the first hour of the reaction after which the rate of the reaction slows considerably.

TABLE 3: TIME COURSE OF IMMOBILIZATION REACTIONS

Time (h)	Relative Intensity
0.0 h	0.0
0.5 h	2.2
1.0 h	3.4
2.0 h	3.9
3.0 h	4.5
4.0 h	5.0
5.0 h	5.4

Example 6: Hybridization Reactions

Hybridization, the formation of a double helix from two oligonucleotides, is a reversible process. Hybridization is dependent upon ionic strength, base composition, the length of the double helix, the concentration of the probe, the concentration of target oligonucleotides and the concentration of helix destabilizing agents. The stability of a duplex formed between strands with mismatched bases is affected by the number and location of mismatches. For oligonucleotides, the T_m decreases by approximately 5 C for every mismatched base pair. The greater number of mismatches, the easier the

sequence discrimination between the matched and imperfectly matched oligonucleotides. The middle position of the mismatch is preferred for better differentiation. Assays are performed under the most stringent hybridization and/or washing conditions to distinguish matched oligonucleotides from those having one base mismatch. Hybridization stringency can be adjusted by varying the salt concentration, the concentration of destablizing agents such as SDS and/or formamide, and/or by changing the temperature of the hybridization reactions.

The degree of discrimination can also be enhanced by adjusting the post hybridization washes. For example, the hybridization can be performed at low stringency and washed a number times, using either the same elution solution or different ones with the increasing stringencies, and the signal intensity measured after each wash.

A. Time Course: In order to confirm hybridization of target oligonucleotide to the probe prepared according to the previous Examples, the following experiment was performed. One pmole of probe P11, *i.e.*,

3'-CGCAAAGAGAAGTTTTTTTTTTTTTTT(CH$_2$)$_6$NH$_2$-5' SEQ ID NO:26

immobilized to glass slides was contacted with 1 pmole of target oligonucleotide D11, *i.e.*,

5'-GCGTTTCTCTTCTTCTTGCG-3' SEQ ID NO:12

labeled at its 5' end with [γ-^{35}S]dATP and T4 nucleotidyl kinase (see Example 5). Hybridization was carried out in 3x SSPE buffer for various times. The probe and the target oligonucleotide hybridize to form the duplex structure:

```
3'-CGCAAAGAGAAGTTTTTTTTTTTTTTT(CH2)6NH2-5'    (SEQ ID NO:26)
    ||||||||||||
5'-GCGTTTCTCTTCTTCTTGCG-3'              D11   (SEQ ID NO:12)
```

As can be seen in Table 4, the hybridization signal reached ½ peak intensity in the first half hour of the reaction and then increased linearly until about 3 hours, at which time the signal remained essentially constant. These results indicate that peak signal intensity is achieved by allowing the hybridization reaction to proceed to equilibrium.

TABLE 4: TIME COURSE OF HYBRIDIZATION REACTIONS

Time (h)	Relative Intensity
0.5 h	3.1
1.0 h	4.6
2.0 h	5.8
3.0 h	7.0
4.0 h	7.0
5.0 h	6.9

B. **Probe Concentration:** The relative concentration of the probes present in the sensor array is another factor affecting the degree of selectivity for specific target (*n*-1) deletion oligonucleotides. The probe concentration should be optimized for specific hybridization of the corresponding matched target oligonucleotide thereto. Target oligonucleotides D3 and D14 (SEQ ID NOS:4 and 15, respectively) were end-labeled with [γ-^{35}S]dATP using T4 nucleotidyl kinase as described in Example 5. Table 5 lists the relative intensity of target oligonucleotides D3 and D14 (SEQ ID NOS:4 and 15, respectively; 0.5 pmol/uL) hybridized to their corresponding matching probes P3 and P14 (SEQ ID NOS:18 and 29, respectively) at different probe concentrations (0 to 7

pmol/uL) for three (3) hours. The relative intensity increases as the probe concentration increases in the 0-0.1 pmol/uL range and remains nearly constant in the 0.1-7.0 pmol/uL range. For best selectivity and highest signal intensity, a probe concentration of 0.5 pmol/uL is preferred. Higher probe concentrations may result in nonspecific binding of mismatched oligonucleotide.

TABLE 5: EFFECT OF PROBE CONCENTRATION ON HYBRIDIZATION (DUPLEX FORMATION)

Probe Conc. (pmol/uL)	Duplex of P3 & D3		Duplex of P14
	Relative Intensity	Error Bar	Relative Intensity
0.000	0.00	0.20	0.00
0.005	0.38	0.20	---
0.010	1.90	0.20	1.2
0.020	4.00	0.30	3.4
0.050	7.25	0.35	5.9
0.10	9.65	0.25	10.2
0.5	10.10	0.20	10.5
1.0	10.20	0.30	10.0
1.5	9.85	0.25	10.0
2.0	9.65	0.55	9.1
3.0	10.00	0.80	9.4
5.0	---	---	10.1
7.0	9.35	1.15	9.5

C. Target Oligonucleotide Concentration: Another factor affecting the degree of selectivity for specific target (n-1) deletion oligonucleotides is the relative concentration of the

target (n-1) deletion oligonucleotides. The target oligonucleotide concentration should be optimized for specific hybridization to the corresponding matched oligonucleotide probe. Target oligonucleotide D3 (SEQ ID NOS:4) was labeled with [γ-^{35}S]dATP using T4 nucleotidyl kinase as described in Example 5. Table 6 shows the relative intensity of different concentrations (0.1 to 4 pmol) of target oligonucleotide D3 (SEQ ID NO:4) hybridized to its corresponding matching probe P3 (SEQ ID NO:18) at three probe concentrations (0.2, 1 and 2 pmol) for three (3) hours. The data in Table 6 show that there is a linear relationship between the signal intensity and the target oligonucleotide concentration when the probe is not saturated. That is, a relatively high concentration of probe (2 uL of 1 pmol/uL) has better linearity for the quantitation of the hybridization reactions.

TABLE 6: EFFECT OF TARGET OLIGONUCLEOTIDE CONCENTRATION ON HYBRIDIZATION

	P3 = 0.2	pmol	P3 = 1.0	pmol	P3 = 2.0	pmol
D3 (pmol)	Relative Intensity	Error Bar	Relative Intensity	Error Bar	Relative Intensity	Error Bar
0.1	1.00	0.09	1.00	0.10	1.00	0.13
0.2	1.71	0.17	1.54	0.13	2.53	0.13
0.4	2.37	0.23	4.25	0.30	6.47	0.27
1.0	2.33	0.17	6.00	0.33	8.67	0.40
2.0	2.40	0.14	6.25	0.50	11.8	0.20
4.0	2.37	0.14	5.82	0.57	12.7	0.47

D. Effect of Temperature: Another factor affecting nucleic acid hybridization reactions is temperature. In order to examine the effect of temperature on nucleic acid hybridization reactions in the present invention, target oligonucleotides D3 (SEQ ID NO:4) and D14 (SEQ ID NO:15) were

labeled as in Example 5 and hybridized to their cognate probe oligonucleotides, P3 (SEQ ID NO:18) and P14 (SEQ ID NO:29), respectively, at various temperatures (30 to 50 C) for three (3) hours. The relative intensity of hybridized material, as determined by scanning the autoradiograph of the reactions on a 300S Molecular Dynamics densitometer is shown in Table 7. The results demonstrate that selectivity increases with increasing temperature until about 45 C. As the temperature rises to greater than 50 C, the melting temperature (T_m) of the perfectly matched duplex is exceeded, resulting in a decrease in hyrbidization efficiency. In this instance, the optimum temperature range for the hybridization reactions is 42° ± 2°C.

TABLE 7: EFFECT OF TEMPERATURE ON HYBRIDIZATION (DUPLEX FORMATION)

	Duplex of P3	& D3	Duplex of P14	& D14
Temperature (°C)	**Relative Intensity**	**Error Bar**	**Relative Intensity**	**Error Bar**
30	5.2	0.42	10.0	0.71
35	3.9	0.15	8.0	0.71
40	3.0	0.14	7.0	0.42
45	1.5	0.09	5.9	0.77
50	1.1	0.31	4.2	0.82

E. Suppression of Mismatched Hybridization by Unlabeled Matched Oligonucleotide: Unlabeled or "cold" target oligonucleotide can be used to suppress hybridization of labeled or "hot" target oligonucleotide to a mismatched probe. In order to assess the suppressive ability of cold target

oligonucleotide D3 (SEQ ID NO:4), the following experiment was carried out. Target oligonucleotide D3 (SEQ ID NO:4) and D14 (SEQ ID NO:15) were labeled as described in Example 5. Both target oligonucleotides were hybridized to probe P14 (SEQ ID NO:29) which is complementary to D14 but which mismatched to D3. Cold D3 was added over a concentration range of 0 to 10 pmol to both hybridization reactions (*i.e.*, D14:P14 and D3:P14). The hybridization reactions took place in 3x SSPE buffer (0.5% SDS) at 30 C for three (3) hours.

The results (Table 8) show that cold D3 had little effect on the hybridization of the matched duplex D14:P14. In contrast, the addition of cold D3 resulted in considerable reduction in the relative radioisotopic intensity for the mismatched duplex D3:P14.

TABLE 8: EFFECT OF UNLABELED TARGET OLIGONUCLEOTIDE ON HYBRIDIZATION SPECIFICITY

Unlabeled D3 (pmol)	Signal from Labeled D14	Interference from Labeled D3	% Interference
0.0	10.00	2.48	24.8
1.0	9.23	1.83	19.8
2.5	9.23	0.95	10.3
3.5	8.46	0.90	10.6
6.0	8.57	0.58	6.71
10.0	9.12	0.35	3.84

The data represented in Table 8 were replotted as % interference (*i.e.*, the ratio, expressed as a percentage, of the relative intensity of the mismatched D3:P14 duplex to that of the perfectly matched D14:P14 duplex). The results demonstrate that the interference by labeled target oligonucleotide D3 (mismatched) of the matched D14:P14 hybridization reaction was reduced from 25% to 5% by the

addition of 10 pmol/uL of unlabeled target oligonucleotide D3 (Table 8).

Example 7: Sequence Specificity of Probes

In order to evaluate the selectivity of the probes of the invention for their cognate probes, the following experiments were done. A matrix of sensor arrays comprising unlabeled probes P1 to P14 (SEQ ID NOS:16 to 29) was constructed on a glass slide according to the method of Examples 1 and 2. Next, target (*n*-1) oligonucleotides D5 (SEQ ID NO:4) and D7 (SEQ ID NO:6) were 5' end-labeled with ^{35}S and T4 nucleotidyl kinase according to the methods described in Example 5. Finally, the target oligonucleotides were hybridized to separate sensor arrays. After allowing the hybridization reactions to proceed to equilibrium at 45 C for 3 hours, the microscope slides comprising the sensor arrays were washed with 2x SSPE buffer solution for 20 minutes and dried. The sensor arrays, to which labeled target oligonucleotides were hybridized, were exposed to AIF film (Fisher Scientific, Pittsburgh, PA) which was then developed according to methods well-known in the art. The autoradiographs were scanned by a densitometer (Molecular Dynamics, Sunnyvale, CA).

The results for target oligonucleotides D5 (SEQ ID NO:4) and D7 (SEQ ID NO:6) are shown in Table 9. In Table 9, the relative intensity of hybridization signal is indicated for probes P1 to P14. The selectivity in specific hybridization is reflected in the fact that the clear majority of target oligonucleotide D5 hybridizes to matched probe P5 (SEQ ID NO:20). In like fashion, the clear majority of target oligonucleotide D7 hybridizes to matched probe P7 (SEQ ID NO:22).

TABLE 9: SPECIFICITY OF TARGET OLIGONUCLEOTIDES

D5 AND D7 FOR PROBES P5 AND P7

Probe	SEQ ID NO:	Relative Intensity of Labeled D5 (SEQ ID NO:6) Bound to Probe	Relative Intensity of Labeled D7 (SEQ ID NO:8) Bound to Probe
P1	16	0.0	0.0
P2	17	0.0	0.0
P3	18	0.0	0.0
P4	19	1.0	0.0
P5	20	11.5	1.0
P6	21	1.4	1.2
P7	22	0.0	11.7
P8	23	0.0	0.0
P9	24	0.0	0.0
P10	25	0.0	0.0
P11	26	1.1	1.0
P12	27	1.9	2.8
P13	28	1.2	0.0

Example 8: Evaluation of (*n*-1) Samples from Different Lots of ISIS 2922

Two lots (referred to herein as lots "A" and "B") of ISIS 2922 (SEQ ID NO:1) were evaluated by the methods and compositions of the disclosure in order to examine the invention's ability to evaluate the composition of (*n*-1) target oligonucleotide compositions from different (*n*-mer) oligonucleotide syntheses. The (*n*-1) target oligonucleotide populations were isolated from the oligonucleotide BDS (bulk drug substance) by cutting the (*n*-1) band of each lot out of a polyacrylamide gel and then subjecting the isolated bands to

freezing and thawing. The (n-1) mixture was further purified and concentrated by ethanol/acetate precipitation according to methods known in the art. The (n-1) target oligonucleotide populations were 5' end-labeled with [γ-^{35}S] dATP and T4 nucleotidyl kinase according to the methods described in Example 5 and hybridized to the sensor array comprising probes P1 to P14 (SEQ ID NOS:16 to 29; see Examples 1 and 7). As can be seen in the Results (Table 10), the matrix and method of the invention detected significant differences in the relative amounts of the indicated (n-1) deletion products. For example, lot "A" has a relative intensity of 2.72 for (n-1) target oligonucleotide D5 (SEQ ID NO:4), whereas lot "B" has a relative intensity of 1.91 for D5. As another example, lot "A" has a relative intensity of 0.58 for (n-1) target oligonucleotide D8 (SEQ ID NO:9), whereas lot "B" has a relative intensity of 0.96 for D8.

These results demonstrate the capacity of the methods and compositions of the invention to measure the relative amounts of each (n-1) target oligonucleotide in the (n-1) subpopulation derived from a synthetic n-mer oligonucleotide. Absolute concentrations can be determined, for example, by the following method.

First, the total amount of nucleic acid material (*i.e*, both n-mer and (n-1) oligonucleotides, as well as other deletion sequences) in the oligonucleotide preparation is determined by means well known in the art such as, for example, measuring the optical density of the preparation in a spectrometer at an O.D. (optical density) of 260 nm and converting the results to concentrations according to known formulas. As an example,

$$OD_{260}/\text{ml} \quad X \quad 1/20 \quad = \quad \text{mg/ml}.$$

Second, the relative amount of (n-1) material in the

preparation is determined by methods known in the art such as, for example, densitometric scanning an autoradiograph of a radiolabeled sample of the preparation that has been electrophoresed on an acrylamide, or measuring the OD_{260} of aliquots of an unlabeled sample of the preparation that have been separated by HPLC (high performance liquid chromatography) or CGE (capillary gel electrophoresis).

Finally, the absolute amount of a given (*n*-1) oligonucleotide, such as, for example, D5 (SEQ ID NO:4) can be determined by the formula:

$$RA_{D5} \times RA_{(n-1)} \times [OLI] = [D5]$$

wherein:

"RA_{D5}" represents the relative amount of (*n*-1) oligonucleotide D5 in the (*n*-1) subpopulation;

"$RA_{(n-1)}$" represents the relative amount of all (*n*-1) oligonucleotides in the *n*-mer preparation;

"[OLI]" represents the absolute amount of all oligonucleotides in the *n*-mer preparation; and

"[D5]" represents the absolute amount of (*n*-1) oligonucleotide D5 in the *n*-mer preparation.

TABLE 10: CHARACTERIZATION OF (*n*-1) POPULATIONS

IN TWO DIFFERENT LOTS OF ISIS 2922

Target No.	SEQ ID NO:	Relative Ratio of (n-1)-mer in... Lot "A"	S.D.	Lot "B"	S.D.
D1	2	0.97	0.13	1.01	0.09
D2	3	0.70	0.13	0.91	0.09
D3	4	2.20	0.35	2.31	0.26
D4	5	1.01	0.13	1.24	0.10
D5	6	2.72	0.50	1.91	0.08
D6	7	0.77	0.20	1.01	0.13
D7	8	2.33	0.52	2.27	0.12
D8	9	0.58	0.19	0.96	0.03
D9	10	1.01	0.25	1.09	0.05
D10	11	0.95	0.13	1.04	0.21
D11	12	1.06	0.11	1.18	0.15
D12	13	2.66	0.10	2.29	0.24
D13	14	1.19	0.06	1.10	0.06
D14	15	0.85	0.13	0.67	0.09

Example 9: Parent Oligonucleotide Sequences

The invention relates to compositions and methods for the identification and characterization of (*n*-1) deletion sequence oligonucleotides in a mixture comprising a synthetic oligonucleotide of length n. In a preferred embodiment, the synthetic oligonucleotide of length n has biological activity and is designed to be administered to cultured cells, isolated tissues and organs and animals. By "biological activity," it is meant that the oligonucleotide functions to modulate the expression of one or more genes in cultured cells, isolated tissues or organs and/or animals. Such modulation can be achieved by an antisense oligonucleotide by a variety of mechanisms known in the art, including but not limited to

transcriptional arrest; effects on RNA processing (capping, polyadenylation and splicing) and transportation; enhancement of cellular degradation of the target nucleic acid; and translational arrest (Crooke *et al.*, *Exp. Opin. Ther. Patents*, 1996, *6*:855).

In an animal other than a human, the compositions and methods of the invention can be used to study the function of one or more genes in the animal. For example, antisense oligonucleotides have been systemically administered to rats in order to study the role of the *N*-methyl-D-aspartate receptor in neuronal death, to mice in order to investigate the biological role of protein kinase C-α, and to rats in order to examine the role of the neuropeptide Y1 receptor in anxiety (Wahlestedt *et al.*, *Nature*, 1993, *363*:260; Dean *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 1994, *91*:11762; and Wahlestedt *et al.*, *Science*, 1993, *259*:528, respectively). In instances where complex families of related proteins are being investigated, "antisense knockouts" (*i.e.*, inhibition of a gene by systemic administration of antisense oligonucleotides) may represent the most accurate means for examining a specific member of the family (see, generally, Albert *et al.*, *Trends Pharmacol. Sci.*, 1994, *15*:250).

The compositions and methods of the invention also have therapeutic uses in an animal, including a human, having (*i.e.*, suffering from), or known to be or suspected of being prone to having, a disease or disorder that is treatable in whole or in part with one or more nucleic acids. The term "therapeutic uses" is intended to encompass prophylactic, palliative and curative uses wherein the oligonucleotides of the invention are contacted with animal cells either *in vivo* or *ex vivo*. When contacted with animal cells *ex vivo*, a therapeutic use includes incorporating such cells into an animal after treatment with one or more oligonucleotides of the invention.

Therapeutic uses are exemplified by the fact that workers in the field have identified antisense, triplex and other oligonucleotide compositions which are capable of modulating expression of genes implicated in viral, fungal and metabolic diseases. Antisense oligonucleotides have been safely administered to humans and several clinical trials are presently underway. It is thus established that oligonucleotides can be useful therapeutic instrumentalities that can be configured to be useful in treatment regimes for treatment of cells, tissues and animals, especially humans. The following U.S. patents demonstrate palliative, therapeutic and other methods utilizing antisense oligonucleotides. U. S. Patent No. 5,135,917 provides antisense oligonucleotides that inhibit human interleukin-1 receptor expression. U.S. Patent No. 5,098,890 is directed to antisense oligonucleotides complementary to the *c-myb* oncogene and antisense oligonucleotide therapies for certain cancerous conditions. U.S. Patent No. 5,087,617 provides methods for treating cancer patients with antisense oligonucleotides.

U.S. Patent No. 5,166,195 provides oligonucleotide inhibitors of Human Immunodeficiency Virus (HIV). U.S. Patent No. 5,004,810 provides oligomers capable of hybridizing to herpes simplex virus Vmw65 mRNA and inhibiting replication. U.S. Patent No. 5,194,428 provides antisense oligonucleotides having antiviral activity against influenza virus. U.S. Patent No. 4,806,463 provides antisense oligonucleotides and methods using them to inhibit HTLV-III replication. U.S. Patent No. 5,286,717 provides oligonucleotides having a complementary base sequence to a portion of an oncogene. U.S. Patent No. 5,276,019 and U.S. Patent No. 5,264,423 are directed to phosphorothioate oligonucleotide analogs used to prevent replication of foreign nucleic acids in cells. U.S. Patent No. 4,689,320 is directed to antisense oligonucleotides as antiviral agents specific to cytomegalovirus (CMV). U.S. Patent No. 5,098,890 provides oligonucleotides complementary to at least a portion of the mRNA transcript of the human *c-*

myb gene. U.S. Patent No. 5,242,906 provides antisense oligonucleotides useful in the treatment of latent Epstein-Barr virus (EBV) infections.

All patents and publications cited herein are incorporated by reference.

The following tables list, as exemplars, some preferred oligonucleotides having biological activity to which the compositions and methods of the invention may be applied. Using the disclosure of the invention one skilled in the art could use the oligonucleotide sequences of the following antisense oligonucleotides, given in the Sequence Listing of the present disclosure, to design appropriate probes for the subpopulation of (n-1) deletion sequence oligonucleotides for each of the following antisense oligonucleotides. Such probes can they be attached to a sensor array and used to characterize the (n-1) deletion sequence subpopulation of a preparation of the antisense oligonucleotide according to the methods of invention. Such desired oligonucleotides include, but are not limited to, those designed to modulate cellular adhesion (Table 11). Other oligonucleotides are designed to modulate cellular proliferation (Table 12), or to have biological or therapeutic activity against miscellaneous disorders (Table 13) and diseases resulting from eukaryotic pathogens (Table 14), retroviruses including HIV (human immunodeficiency virus; Table 15) or non-retroviral viral viruses (Table 16). Further details regarding the sources of the following oligonucleotides are provided in the Sequence Listing.

TABLE 11: TARGET OLIGONUCLEOTIDES DESIGNED

TO MODULATE CELLULAR ADHESION

Cell Surface Target Protein	Commercial or Common Name (if any)	Oligonucleotide Sequence SEQ ID NO(S):
ICAM-1	ISIS 2302	31
ICAM-1	GM1595	32
VCAM-1	ISIS 5847	33
VCAM-1	GM1535	34
ELAM-1	GM1515 to GM1517	35, 36, 37

TABLE 12: OLIGONUCLEOTIDES DESIGNED TO MODULATE CELLULAR PROLIFERATION

Molecular Target	Commercial or Common Name (if any)	Oligonucleotide Sequence SEQ ID NO(S):
c-myb	MYB-AS	38
DNA methyl transferase		39, 40
vascular endothelial growth factor (VEGF)		41, 42, 43, 44, 45, 46, 47, 48, 49, 50
VEGF	HS	132
VEGF	Vm	133
bcl-2		134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145
bcl-2	BCL-2	146
bcl-abl		147
PKC-α, -β, -γ & -ζ	$oligo_{antiPKC\alpha}$	148
PKC-α	ISIS 3521	149
PKC-ζ		150
protein kinase A, subunit RI_{α}		151, 152, 153
βARK1 & βARK2	$oligo_{anti\beta ARK2}$	154

Ha-ras	ISIS 2503	155
MDR		156, 157, 158, 159
MRP	ISIS 7597	160
A-raf kinase	ISIS 9069	161
c-raf kinase	ISIS 5132	162

TABLE 13: OLIGONUCLEOTIDES DESIGNED TO HAVE THERAPEUTIC ACTIVITY AGAINST MISCELLANEOUS DISORDERS

Disorder	Commercial/ Common Name (if any)	Oligonucleotide Sequences SEQ ID NO(S):
Alzheimer's disease		51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62
Beta-thalassemia	5'ss & 3'ss	63, 64

TABLE 14: OLIGONUCLEOTIDES DESIGNED TO HAVE BIOLOGICAL ACTIVITY AGAINST EUKARYOTIC PATHOGENS

Pathogen / Disease	Commercial/ Common Name (if any)	Oligonucleotide Sequences SEQ ID NO(S):
Plasmodium / malaria	PSI, PSII PSIII & RI	65, 66, 67, 68
Schistosoma / bloodfluke infections		69

TABLE 15: OLIGONUCLEOTIDES DESIGNED TO HAVE BIOLOGICAL ACTIVITY AGAINST RETROVIRUSES, INCLUDING HIV

Virus / Molecular Target	Commercial/ Common Name (if any)	Oligonucleotide Sequences SEQ ID NO(S):
HTLV-III / primer binding site		70, 71, 72, 73, 74, 75
HIV-1 / *gag*	GEM 91	76
HIV-1 / *gag*	GEM 92, GEM 93	77, 78, 79, 80, 81, 82, 83, 84, 85
HIV	AR 177	86
HIV / *tat, vpr, rev, env, nef*		87, 88, 89
HIV / *pol, env, vir*		90, 91, 92, 93, 94, 95, 96, 97
HIV-1 / *tat, rev, env, nef*		98, 99, 100, 101, 102, 103
HIV / *gp*120	ISIS 5320	104
Hepatitis C virus	ISIS 6547	105

TABLE 16: OLIGONUCLEOTIDES DESIGNED TO HAVE BIOLOGICAL ACTIVITY AGAINST NON-RETROVIRAL VIRUSES

Virus / Molecular Target	Commercial/ Common Name (if any)	Oligonucleotide Sequences SEQ ID NO(S):
influenza virus		106, 107, 108, 109, 110, 111, 112, 113, 114
Epstein-Barr Virus		115, 116, 117
Respiratory Syncytial Virus		118, 119, 120, 121
cytomegalovirus (CMV)	GEM 132	122
CMV		123, 124, 125, 126, 127, 128, 129, 130
CMV	ISIS 2922	131

Example 10: Choice of Probe Oligonucleotide Chemistries

When choosing a probe oligonucleotide chemistry to be used for the characterization of a set of target deletion

sequence oligonucleotides, one important consideration is the chemical nature of the target oligonucleotides. In general, target oligonucleotides are "DNA-like" (i.e., having 2'-deoxy sugars and T rather than U bases) or "RNA-like" (i.e., having 2'-hydroxyl or 2'-modified sugars and U rather than T bases). These criteria are not absolute, that is, an oligonucleotide can comprise a majority of 2'-deoxy sugars and a few 2'-hydroxyl sugars and still be considered "DNA-like" for the purposes of this invention. As detailed herein, some probe oligonucleotide chemistries are preferred for the characterization of DNA-like target oligonucleotides, some are preferred for RNA-like target oligonucleotides, and some function with approximately equal effectiveness for either type of target oligonucleotide.

A. Probe Chemistries for DNA-Like Target Oligonucleotides: DNA-like target oligonucleotides include but are not limited to oligonucleotides that are entirely or predominately oligodeoxynucleotides (ODNs; i.e., 2'-deoxy-oligonucleotides), and/or have the oxygen of the furanosyl group replaced with S or CH_2, and/or have one or more base modifications such as, e.g., 5-methylcytosine (m5c) in lieu of cytosine (C); 2,6-diaminopurine (DAP, also known as 2-aminoadenine) in lieu of adenine (A); 2-aminoguanine in lieu of guanine (G); and hypoxanthine (I) in lieu of any other nucleobase.

Preferred probe oligonucleotides for the hybridization of DNA-like target oligonucleotides are generally other DNA-like oligonucleotides, including but not limited to oligonucleotides having (1) a fully or predominantly phosphodiester backbone, (2) entirely or predominately 2'-deoxy-oligonucleotides, (3) the oxygen of the furanosyl group replaced with S or CH_2, and/or (4) one or more base modifications. Such base modifications include, for example, 2,6-diaminopurine (DAP, also known as 2-aminoadenine) in lieu of adenine (A); 2-aminoguanine (2AG) in lieu of guanine (G); hypoxanthine (I) in lieu of any other nucleobase; 5-(1-

propynyl)uracil (5PU) in lieu of thymine (T); and 5-(1-propynyl)cytosine (5PC) or 5-methylcytosine (m5c) in lieu of cytosine (C).

Probe oligonucleotides having these preferred chemical modifications are synthesized according to the methods and teachings incorporated by reference set forth in Example 2 and in Prosnyak *et al.* (*Genomics*, 1994, *21*, 490; DAP and m5c), Bailly *et al.* (*Proc. Natl. Acad. Sci. U.S.A.*, 1996, *93*, 13263; DAP and I), Chollet *et al.* (*Nucleic Acids Research*, 1988, *16*, 305; DAP) and U.S. Patent 5,645,985 to Froehler *et al.* (5PC and 5PU).

B. Probe Chemistries for RNA-Like Target Oligonucleotides: RNA-like target oligonucleotides include but are not limited to oligonucleotides that are entirely or predominately oligoribonucleotides (i.e., 2'-hydroxy-oligonucleotides), oligonucleotides having a majority of sugars with 2' modifications, and oligonucleotides having a fully or predominately phosphorothioate backbone.

Preferred probe oligonucleotide chemistries for the hybridization of RNA-like target oligonucleotides have been extensively described by Freier *et al.*(*Nucleic Acids Research*, 1997, *25*, 4429). Such preferred probe oligonucleotide chemistries include but are not limited to sugar modifications [such as 2'-fluoro; 2'-*O*-alkyl; 2'-methoxyethoxy; 2'-propyl-O-butyl; 2'-(ethylene glycol)$_{2-4}$; 2'-nonyl; 2'-dimethylaminoethoxy; 2'-dimethylamino-ethoxyethoxy; 2'-monomethylaminoethoxy; 2'-aminoethoxy; 2'-piperazinethoxy; 2'-(3'-N,N-dimethylamino-1-propyl)aminoethoxy; and 2'-O-CH_2-CHR-X, where X is OH, F, CF_3 or OCH_3 and R is independently H, CH_3, CH_2OH or CH_2OCH_3], modified nucleobases (such as 5-propynyl dU; 5-amino-propyl dU; 2-thio T; 2'-*O*-methyl U; 2'-*O*-methyl pseudo U; 7-halo-7-deaza purines; 7-propyne-7-deaza purines; and 2,6-diaminopurine), and backbone modifications [such as thioformacetal, -S-CH_2-O-CH_2-; amide 3 or amide 4 linkages in combination with more flexible linkages; MMI,

methylene(methylimino); MDH, dimethylhydrazino; and N3'-> P5' phosphoramidites], and combinations thereof.

Probe oligonucleotides having these preferred chemical modifications are synthesized according to the methods and teachings incorporated by reference set forth in Example 2 and in published PCT application WO 97/46569, European Patents 0626387 and 0679657, and in copending U.S. patent applications having Serial Nos. 09/115,025(Attorney Docket No. ISIS-2951 filed July 14, 1998); 09/115,027 (Attorney Docket No. ISIS-2953 filed July 14, 1998): 09/066,638 (Attorney Docket No. ISIS-2914 filed April 24, 1998); 60/078,637 (Attorney Docket No. ISIS-2907 filed March 19, 1998); 09/130,973 (Attorney Docket No. ISIS-2955 filed August 7, 1998); 09/123,108 (Attorney Docket No. ISIS-3147 filed July 27, 1998); 09/123,036 (Attorney Docket No. ISIS-3149 filed August 3, 1998); and 09/130,566 (Attorney Docket No. ISIS-3156 filed August 7, 1998),each of which is incorporated herein by reference.

C. "Universal" Probe Chemistries: Peptide nucleic acids (PNAs) have strong hybridization affinities for both DNA-like and RNA-like oligonucleotides. Accordingly, a single composition having PNA probes in its sensor arrays can be used, for example, to characterize the (*n*-1) deletion sequence oligonucleotides present in a preparation of a synthetic hybrid oligonucleotide that comprises both DNA-like and RNA-like portions. Probe peptide nucleic acids are synthesized according to the methods set forth in Example 2.

CLAIMS

What is claimed is:

1. A composition comprising a plurality, x, of sensor arrays, wherein each sensor array comprises a unique probe oligonucleotide having a sequence that is the reverse complement of at least a portion of a unique target oligonucleotide present in a mixture of target deletion sequence oligonucleotides.

2. The composition of claim 1, wherein said sensor arrays are arranged in the form of a matrix.

3. The composition of claim 1, wherein x is from 2 to about 50.

4. The composition of claim 1, wherein x is from 8 to about 50.

5. The composition of claim 1, wherein x is from 8 to 20.

6. The composition of claim 1, wherein said sensor array further comprises a linker.

7. The composition of claim 1, wherein said sensor array further comprises a spacer.

8. The composition of claim 6, wherein said sensor array further comprises a spacer.

9. The composition of claim 1, wherein said probe oligonucleotide of said sensor array is an oligodeoxyribonucleotide, an oligoribonucleotide, a peptide nucleic acid, a chimeric oligonucleotide, an oligonucleotide having one or more modified linkages, an oligonucleotide having one or more modified sugar residues or an oligonucleotide having one or more modified nucleobases.

10. The sensor array of claim 1, wherein said probe oligonucleotides of said sensor arrays have sequences that are the reverse complements of target deletion sequence

labeled as in Example 5 and hybridized to their cognate probe oligonucleotides, P3 (SEQ ID NO:18) and P14 (SEQ ID NO:29), respectively, at various temperatures (30 to 50 C) for three (3) hours. The relative intensity of hybridized material, as determined by scanning the autoradiograph of the reactions on a 300S Molecular Dynamics densitometer is shown in Table 7. The results demonstrate that selectivity increases with increasing temperature until about 45 C. As the temperature rises to greater than 50 C, the melting temperature (T_m) of the perfectly matched duplex is exceeded, resulting in a decrease in hyrbidization efficiency. In this instance, the optimum temperature range for the hybridization reactions is $42^o \pm 2^oC$.

TABLE 7: EFFECT OF TEMPERATURE ON HYBRIDIZATION (DUPLEX FORMATION)

	Duplex of P3	& D3	Duplex of P14	& D14
Temperature (°C)	Relative Intensity	Error Bar	Relative Intensity	Error Bar
30	5.2	0.42	10.0	0.71
35	3.9	0.15	8.0	0.71
40	3.0	0.14	7.0	0.42
45	1.5	0.09	5.9	0.77
50	1.1	0.31	4.2	0.82

E. Suppression of Mismatched Hybridization by Unlabeled Matched Oligonucleotide: Unlabeled or "cold" target oligonucleotide can be used to suppress hybridization of labeled or "hot" target oligonucleotide to a mismatched probe. In order to assess the suppressive ability of cold target

addition of 10 pmol/uL of unlabeled target oligonucleotide D3 (Table 8).

Example 7: Sequence Specificity of Probes

In order to evaluate the selectivity of the probes of the invention for their cognate probes, the following experiments were done. A matrix of sensor arrays comprising unlabeled probes P1 to P14 (SEQ ID NOS:16 to 29) was constructed on a glass slide according to the method of Examples 1 and 2. Next, target (*n*-1) oligonucleotides D5 (SEQ ID NO:4) and D7 (SEQ ID NO:6) were 5' end-labeled with ^{35}S and T4 nucleotidyl kinase according to the methods described in Example 5. Finally, the target oligonucleotides were hybridized to separate sensor arrays. After allowing the hybridization reactions to proceed to equilibrium at 45 C for 3 hours, the microscope slides comprising the sensor arrays were washed with 2x SSPE buffer solution for 20 minutes and dried. The sensor arrays, to which labeled target oligonucleotides were hybridized, were exposed to AIF film (Fisher Scientific, Pittsburgh, PA) which was then developed according to methods well-known in the art. The autoradiographs were scanned by a densitometer (Molecular Dynamics, Sunnyvale, CA).

The results for target oligonucleotides D5 (SEQ ID NO:4) and D7 (SEQ ID NO:6) are shown in Table 9. In Table 9, the relative intensity of hybridization signal is indicated for probes P1 to P14. The selectivity in specific hybridization is reflected in the fact that the clear majority of target oligonucleotide D5 hybridizes to matched probe P5 (SEQ ID NO:20). In like fashion, the clear majority of target oligonucleotide D7 hybridizes to matched probe P7 (SEQ ID NO:22).

TABLE 9: SPECIFICITY OF TARGET OLIGONUCLEOTIDES

D5 AND D7 FOR PROBES P5 AND P7

Probe	SEQ ID NO:	Relative Intensity of Labeled D5 (SEQ ID NO:6) Bound to Probe	Relative Intensity of Labeled D7 (SEQ ID NO:8) Bound to Probe
P1	16	0.0	0.0
P2	17	0.0	0.0
P3	18	0.0	0.0
P4	19	1.0	0.0
P5	20	11.5	1.0
P6	21	1.4	1.2
P7	22	0.0	11.7
P8	23	0.0	0.0
P9	24	0.0	0.0
P10	25	0.0	0.0
P11	26	1.1	1.0
P12	27	1.9	2.8
P13	28	1.2	0.0

Example 8: Evaluation of (*n*-1) Samples from Different Lots of ISIS 2922

Two lots (referred to herein as lots "A" and "B") of ISIS 2922 (SEQ ID NO:1) were evaluated by the methods and compositions of the disclosure in order to examine the invention's ability to evaluate the composition of (*n*-1) target oligonucleotide compositions from different (*n*-mer) oligonucleotide syntheses. The (*n*-1) target oligonucleotide populations were isolated from the oligonucleotide BDS (bulk drug substance) by cutting the (*n*-1) band of each lot out of a polyacrylamide gel and then subjecting the isolated bands to

freezing and thawing. The (n-1) mixture was further purified and concentrated by ethanol/acetate precipitation according to methods known in the art. The (n-1) target oligonucleotide populations were 5' end-labeled with [γ-^{35}S] dATP and T4 nucleotidyl kinase according to the methods described in Example 5 and hybridized to the sensor array comprising probes P1 to P14 (SEQ ID NOS:16 to 29; see Examples 1 and 7). As can be seen in the Results (Table 10), the matrix and method of the invention detected significant differences in the relative amounts of the indicated (n-1) deletion products. For example, lot "A" has a relative intensity of 2.72 for (n-1) target oligonucleotide D5 (SEQ ID NO:4), whereas lot "B" has a relative intensity of 1.91 for D5. As another example, lot "A" has a relative intensity of 0.58 for (n-1) target oligonucleotide D8 (SEQ ID NO:9), whereas lot "B" has a relative intensity of 0.96 for D8.

These results demonstrate the capacity of the methods and compositions of the invention to measure the relative amounts of each (n-1) target oligonucleotide in the (n-1) subpopulation derived from a synthetic n-mer oligonucleotide. Absolute concentrations can be determined, for example, by the following method.

First, the total amount of nucleic acid material (*i.e*, both n-mer and (n-1) oligonucleotides, as well as other deletion sequences) in the oligonucleotide preparation is determined by means well known in the art such as, for example, measuring the optical density of the preparation in a spectrometer at an O.D. (optical density) of 260 nm and converting the results to concentrations according to known formulas. As an example,

$$OD_{260}/ml \times 1/20 = mg/ml.$$

Second, the relative amount of (n-1) material in the

preparation is determined by methods known in the art such as, for example, densitometric scanning an autoradiograph of a radiolabeled sample of the preparation that has been electrophoresed on an acrylamide, or measuring the OD_{260} of aliquots of an unlabeled sample of the preparation that have been separated by HPLC (high performance liquid chromatography) or CGE (capillary gel electrophoresis).

Finally, the absolute amount of a given (n-1) oligonucleotide, such as, for example, D5 (SEQ ID NO:4) can be determined by the formula:

$$RA_{D5} \times RA_{(n-1)} \times [OLI] = [D5]$$

wherein:

"RA_{D5}" represents the relative amount of (n-1) oligonucleotide D5 in the (n-1) subpopulation;

"$RA_{(n-1)}$" represents the relative amount of all (n-1) oligonucleotides in the n-mer preparation;

"[OLI]" represents the absolute amount of all oligonucleotides in the n-mer preparation; and

"[D5]" represents the absolute amount of (n-1) oligonucleotide D5 in the n-mer preparation.

TABLE 10: CHARACTERIZATION OF (n-1) POPULATIONS

IN TWO DIFFERENT LOTS OF ISIS 2922

Target No.	SEQ ID NO:	*Relative Ratio of (n-1)-mer in...* Lot "A"	S.D.	Lot "B"	S.D.
D1	2	0.97	0.13	1.01	0.09
D2	3	0.70	0.13	0.91	0.09
D3	4	2.20	0.35	2.31	0.26
D4	5	1.01	0.13	1.24	0.10
D5	6	2.72	0.50	1.91	0.08
D6	7	0.77	0.20	1.01	0.13
D7	8	2.33	0.52	2.27	0.12
D8	9	0.58	0.19	0.96	0.03
D9	10	1.01	0.25	1.09	0.05
D10	11	0.95	0.13	1.04	0.21
D11	12	1.06	0.11	1.18	0.15
D12	13	2.66	0.10	2.29	0.24
D13	14	1.19	0.06	1.10	0.06
D14	15	0.85	0.13	0.67	0.09

Example 9: Parent Oligonucleotide Sequences

The invention relates to compositions and methods for the identification and characterization of (*n*-1) deletion sequence oligonucleotides in a mixture comprising a synthetic oligonucleotide of length n. In a preferred embodiment, the synthetic oligonucleotide of length n has biological activity and is designed to be administered to cultured cells, isolated tissues and organs and animals. By "biological activity," it is meant that the oligonucleotide functions to modulate the expression of one or more genes in cultured cells, isolated tissues or organs and/or animals. Such modulation can be achieved by an antisense oligonucleotide by a variety of mechanisms known in the art, including but not limited to

transcriptional arrest; effects on RNA processing (capping, polyadenylation and splicing) and transportation; enhancement of cellular degradation of the target nucleic acid; and translational arrest (Crooke *et al.*, *Exp. Opin. Ther. Patents*, 1996, *6*:855).

In an animal other than a human, the compositions and methods of the invention can be used to study the function of one or more genes in the animal. For example, antisense oligonucleotides have been systemically administered to rats in order to study the role of the *N*-methyl-D-aspartate receptor in neuronal death, to mice in order to investigate the biological role of protein kinase C-α, and to rats in order to examine the role of the neuropeptide Y1 receptor in anxiety (Wahlestedt *et al.*, *Nature*, 1993, *363*:260; Dean *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 1994, *91*:11762; and Wahlestedt *et al.*, *Science*, 1993, *259*:528, respectively). In instances where complex families of related proteins are being investigated, "antisense knockouts" (*i.e.*, inhibition of a gene by systemic administration of antisense oligonucleotides) may represent the most accurate means for examining a specific member of the family (see, generally, Albert *et al.*, *Trends Pharmacol. Sci.*, 1994, *15*:250).

The compositions and methods of the invention also have therapeutic uses in an animal, including a human, having (*i.e.*, suffering from), or known to be or suspected of being prone to having, a disease or disorder that is treatable in whole or in part with one or more nucleic acids. The term "therapeutic uses" is intended to encompass prophylactic, palliative and curative uses wherein the oligonucleotides of the invention are contacted with animal cells either *in vivo* or *ex vivo*. When contacted with animal cells *ex vivo*, a therapeutic use includes incorporating such cells into an animal after treatment with one or more oligonucleotides of the invention.

Therapeutic uses are exemplified by the fact that workers in the field have identified antisense, triplex and other oligonucleotide compositions which are capable of modulating expression of genes implicated in viral, fungal and metabolic diseases. Antisense oligonucleotides have been safely administered to humans and several clinical trials are presently underway. It is thus established that oligonucleotides can be useful therapeutic instrumentalities that can be configured to be useful in treatment regimes for treatment of cells, tissues and animals, especially humans. The following U.S. patents demonstrate palliative, therapeutic and other methods utilizing antisense oligonucleotides. U. S. Patent No. 5,135,917 provides antisense oligonucleotides that inhibit human interleukin-1 receptor expression. U.S. Patent No. 5,098,890 is directed to antisense oligonucleotides complementary to the *c-myb* oncogene and antisense oligonucleotide therapies for certain cancerous conditions. U.S. Patent No. 5,087,617 provides methods for treating cancer patients with antisense oligonucleotides.

U.S. Patent No. 5,166,195 provides oligonucleotide inhibitors of Human Immunodeficiency Virus (HIV). U.S. Patent No. 5,004,810 provides oligomers capable of hybridizing to herpes simplex virus Vmw65 mRNA and inhibiting replication. U.S. Patent No. 5,194,428 provides antisense oligonucleotides having antiviral activity against influenza virus. U.S. Patent No. 4,806,463 provides antisense oligonucleotides and methods using them to inhibit HTLV-III replication. U.S. Patent No. 5,286,717 provides oligonucleotides having a complementary base sequence to a portion of an oncogene. U.S. Patent No. 5,276,019 and U.S. Patent No. 5,264,423 are directed to phosphorothioate oligonucleotide analogs used to prevent replication of foreign nucleic acids in cells. U.S. Patent No. 4,689,320 is directed to antisense oligonucleotides as antiviral agents specific to cytomegalovirus (CMV). U.S. Patent No. 5,098,890 provides oligonucleotides complementary to at least a portion of the mRNA transcript of the human *c-*

myb gene. U.S. Patent No. 5,242,906 provides antisense oligonucleotides useful in the treatment of latent Epstein-Barr virus (EBV) infections.

All patents and publications cited herein are incorporated by reference.

The following tables list, as exemplars, some preferred oligonucleotides having biological activity to which the compositions and methods of the invention may be applied. Using the disclosure of the invention one skilled in the art could use the oligonucleotide sequences of the following antisense oligonucleotides, given in the Sequence Listing of the present disclosure, to design appropriate probes for the subpopulation of (*n*-1) deletion sequence oligonucleotides for each of the following antisense oligonucleotides. Such probes can they be attached to a sensor array and used to characterize the (*n*-1) deletion sequence subpopulation of a preparation of the antisense oligonucleotide according to the methods of invention. Such desired oligonucleotides include, but are not limited to, those designed to modulate cellular adhesion (Table 11). Other oligonucleotides are designed to modulate cellular proliferation (Table 12), or to have biological or therapeutic activity against miscellaneous disorders (Table 13) and diseases resulting from eukaryotic pathogens (Table 14), retroviruses including HIV (human immunodeficiency virus; Table 15) or non-retroviral viral viruses (Table 16). Further details regarding the sources of the following oligonucleotides are provided in the Sequence Listing.

TABLE 11: TARGET OLIGONUCLEOTIDES DESIGNED

TO MODULATE CELLULAR ADHESION

Cell Surface Target Protein	Commercial or Common Name (if any)	Oligonucleotide Sequence SEQ ID NO(S):
ICAM-1	ISIS 2302	31
ICAM-1	GM1595	32
VCAM-1	ISIS 5847	33
VCAM-1	GM1535	34
ELAM-1	GM1515 to GM1517	35, 36, 37

TABLE 12: OLIGONUCLEOTIDES DESIGNED TO MODULATE CELLULAR PROLIFERATION

Molecular Target	Commercial or Common Name (if any)	Oligonucleotide Sequence SEQ ID NO(S):
c-myb	MYB-AS	38
DNA methyl transferase		39, 40
vascular endothelial growth factor (VEGF)		41, 42, 43, 44, 45, 46, 47, 48, 49, 50
VEGF	HS	132
VEGF	Vm	133
bcl-2		134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145
bcl-2	BCL-2	146
bcl-abl		147
PKC-α, -β, -γ & -ζ	$oligo_{antiPKCα}$	148
PKC-α	ISIS 3521	149
PKC-ζ		150
protein kinase A, subunit $RI_α$		151, 152, 153
βARK1 & βARK2	$oligo_{antiβARK2}$	154

Ha-ras	ISIS 2503	155
MDR		156, 157, 158, 159
MRP	ISIS 7597	160
A-raf kinase	ISIS 9069	161
c-raf kinase	ISIS 5132	162

TABLE 13: OLIGONUCLEOTIDES DESIGNED TO HAVE THERAPEUTIC ACTIVITY AGAINST MISCELLANEOUS DISORDERS

Disorder	Commercial/ Common Name (if any)	Oligonucleotide Sequences SEQ ID NO(S):
Alzheimer's disease		51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62
Beta-thalassemia	5'ss & 3'ss	63, 64

TABLE 14: OLIGONUCLEOTIDES DESIGNED TO HAVE BIOLOGICAL ACTIVITY AGAINST EUKARYOTIC PATHOGENS

Pathogen / Disease	Commercial/ Common Name (if any)	Oligonucleotide Sequences SEQ ID NO(S):
Plasmodium / malaria	PSI, PSII PSIII & RI	65, 66, 67, 68
Schistosoma / bloodfluke infections		69

TABLE 15: OLIGONUCLEOTIDES DESIGNED TO HAVE BIOLOGICAL ACTIVITY AGAINST RETROVIRUSES, INCLUDING HIV

Virus / Molecular Target	Commercial/ Common Name (if any)	Oligonucleotide Sequences SEQ ID NO(S):
HTLV-III / primer binding site		70, 71, 72, 73, 74, 75
HIV-1 / *gag*	GEM 91	76
HIV-1 / *gag*	GEM 92, GEM 93	77, 78, 79, 80, 81, 82, 83, 84, 85
HIV	AR 177	86
HIV / *tat, vpr, rev, env, nef*		87, 88, 89
HIV / *pol, env, vir*		90, 91, 92, 93, 94, 95, 96, 97
HIV-1 / *tat, rev, env, nef*		98, 99, 100, 101, 102, 103
HIV / *gp*120	ISIS 5320	104
Hepatitis C virus	ISIS 6547	105

TABLE 16: OLIGONUCLEOTIDES DESIGNED TO HAVE BIOLOGICAL ACTIVITY AGAINST NON-RETROVIRAL VIRUSES

Virus / Molecular Target	Commercial/ Common Name (if any)	Oligonucleotide Sequences SEQ ID NO(S):
influenza virus		106, 107, 108, 109, 110, 111, 112, 113, 114
Epstein-Barr Virus		115, 116, 117
Respiratory Syncytial Virus		118, 119, 120, 121
cytomegalovirus (CMV)	GEM 132	122
CMV		123, 124, 125, 126, 127, 128, 129, 130
CMV	ISIS 2922	131

Example 10: Choice of Probe Oligonucleotide Chemistries

When choosing a probe oligonucleotide chemistry to be used for the characterization of a set of target deletion

sequence oligonucleotides, one important consideration is the chemical nature of the target oligonucleotides. In general, target oligonucleotides are "DNA-like" (i.e., having 2'-deoxy sugars and T rather than U bases) or "RNA-like" (i.e., having 2'-hydroxyl or 2'-modified sugars and U rather than T bases). These criteria are not absolute, that is, an oligonucleotide can comprise a majority of 2'-deoxy sugars and a few 2'-hydroxyl sugars and still be considered "DNA-like" for the purposes of this invention. As detailed herein, some probe oligonucleotide chemistries are preferred for the characterization of DNA-like target oligonucleotides, some are preferred for RNA-like target oligonucleotides, and some function with approximately equal effectiveness for either type of target oligonucleotide.

A. Probe Chemistries for DNA-Like Target Oligonucleotides: DNA-like target oligonucleotides include but are not limited to oligonucleotides that are entirely or predominately oligodeoxynucleotides (ODNs; i.e., 2'-deoxy-oligonucleotides), and/or have the oxygen of the furanosyl group replaced with S or CH_2, and/or have one or more base modifications such as, e.g., 5-methylcytosine (m5c) in lieu of cytosine (C); 2,6-diaminopurine (DAP, also known as 2-aminoadenine) in lieu of adenine (A); 2-aminoguanine in lieu of guanine (G); and hypoxanthine (I) in lieu of any other nucleobase.

Preferred probe oligonucleotides for the hybridization of DNA-like target oligonucleotides are generally other DNA-like oligonucleotides, including but not limited to oligonucleotides having (1) a fully or predominantly phosphodiester backbone, (2) entirely or predominately 2'-deoxy-oligonucleotides, (3) the oxygen of the furanosyl group replaced with S or CH_2, and/or (4) one or more base modifications. Such base modifications include, for example, 2,6-diaminopurine (DAP, also known as 2-aminoadenine) in lieu of adenine (A); 2-aminoguanine (2AG) in lieu of guanine (G); hypoxanthine (I) in lieu of any other nucleobase; 5-(1-

propynyl)uracil (5PU) in lieu of thymine (T); and 5-(1-propynyl)cytosine (5PC) or 5-methylcytosine (m5c) in lieu of cytosine (C).

Probe oligonucleotides having these preferred chemical modifications are synthesized according to the methods and teachings incorporated by reference set forth in Example 2 and in Prosnyak *et al.* (*Genomics*, 1994, *21*, 490; DAP and m5c), Bailly *et al.* (*Proc. Natl. Acad. Sci. U.S.A.*, 1996, *93*, 13263; DAP and I), Chollet *et al.* (*Nucleic Acids Research*, 1988, *16*, 305; DAP) and U.S. Patent 5,645,985 to Froehler *et al.* (5PC and 5PU).

B. Probe Chemistries for RNA-Like Target Oligonucleotides: RNA-like target oligonucleotides include but are not limited to oligonucleotides that are entirely or predominately oligoribonucleotides (i.e., 2'-hydroxy-oligonucleotides), oligonucleotides having a majority of sugars with 2' modifications, and oligonucleotides having a fully or predominately phosphorothioate backbone.

Preferred probe oligonucleotide chemistries for the hybridization of RNA-like target oligonucleotides have been extensively described by Freier *et al.*(*Nucleic Acids Research*, 1997, *25*, 4429). Such preferred probe oligonucleotide chemistries include but are not limited to sugar modifications [such as 2'-fluoro; 2'-*O*-alkyl; 2'-methoxyethoxy; 2'-propyl-O-butyl; 2'-(ethylene glycol)$_{2-4}$; 2'-nonyl; 2'-dimethylaminoethoxy; 2'-dimethylamino-ethoxyethoxy; 2'-monomethylaminoethoxy; 2'-aminoethoxy; 2'-piperazinethoxy; 2'-(3'-N,N-dimethylamino-1-propyl)aminoethoxy; and 2'-O-CH_2-CHR-X, where X is OH, F, CF_3 or OCH_3 and R is independently H, CH_3, CH_2OH or CH_2OCH_3], modified nucleobases (such as 5-propynyl dU; 5-amino-propyl dU; 2-thio T; 2'-*O*-methyl U; 2'-*O*-methyl pseudo U; 7-halo-7-deaza purines; 7-propyne-7-deaza purines; and 2,6-diaminopurine), and backbone modifications [such as thioformacetal, -S-CH_2-O-CH_2-; amide 3 or amide 4 linkages in combination with more flexible linkages; MMI,

methylene(methylimino); MDH, dimethylhydrazino; and N3'-> P5' phosphoramidites], and combinations thereof.

Probe oligonucleotides having these preferred chemical modifications are synthesized according to the methods and teachings incorporated by reference set forth in Example 2 and in published PCT application WO 97/46569, European Patents 0626387 and 0679657, and in copending U.S. patent applications having Serial Nos. 09/115,025(Attorney Docket No. ISIS-2951 filed July 14, 1998); 09/115,027 (Attorney Docket No. ISIS-2953 filed July 14, 1998): 09/066,638 (Attorney Docket No. ISIS-2914 filed April 24, 1998); 60/078,637 (Attorney Docket No. ISIS-2907 filed March 19, 1998); 09/130,973 (Attorney Docket No. ISIS-2955 filed August 7, 1998); 09/123,108 (Attorney Docket No. ISIS-3147 filed July 27, 1998); 09/123,036 (Attorney Docket No. ISIS-3149 filed August 3, 1998); and 09/130,566 (Attorney Docket No. ISIS-3156 filed August 7, 1998),each of which is incorporated herein by reference.

C. "Universal" Probe Chemistries: Peptide nucleic acids (PNAs) have strong hybridization affinities for both DNA-like and RNA-like oligonucleotides. Accordingly, a single composition having PNA probes in its sensor arrays can be used, for example, to characterize the (*n*-1) deletion sequence oligonucleotides present in a preparation of a synthetic hybrid oligonucleotide that comprises both DNA-like and RNA-like portions. Probe peptide nucleic acids are synthesized according to the methods set forth in Example 2.

CLAIMS

What is claimed is:

1. A composition comprising a plurality, x, of sensor arrays, wherein each sensor array comprises a unique probe oligonucleotide having a sequence that is the reverse complement of at least a portion of a unique target oligonucleotide present in a mixture of target deletion sequence oligonucleotides.

2. The composition of claim 1, wherein said sensor arrays are arranged in the form of a matrix.

3. The composition of claim 1, wherein x is from 2 to about 50.

4. The composition of claim 1, wherein x is from 8 to about 50.

5. The composition of claim 1, wherein x is from 8 to 20.

6. The composition of claim 1, wherein said sensor array further comprises a linker.

7. The composition of claim 1, wherein said sensor array further comprises a spacer.

8. The composition of claim 6, wherein said sensor array further comprises a spacer.

9. The composition of claim 1, wherein said probe oligonucleotide of said sensor array is an oligodeoxyribonucleotide, an oligoribonucleotide, a peptide nucleic acid, a chimeric oligonucleotide, an oligonucleotide having one or more modified linkages, an oligonucleotide having one or more modified sugar residues or an oligonucleotide having one or more modified nucleobases.

10. The sensor array of claim 1, wherein said probe oligonucleotides of said sensor arrays have sequences that are the reverse complements of target deletion sequence

oligonucleotides of length n-1 derived from a synthetic oligonucleotide of length n.

11. The sensor array of claim 10, wherein said synthetic oligonucleotide is designed to modulate cellular adhesion or cellular proliferation.

12. The sensor array of claim 10, wherein said synthetic oligonucleotide is an oligonucleotide designed to have biological activity against a eukaryotic pathogen.

13. The sensor array of claim 10, wherein said synthetic oligonucleotide is an oligonucleotide designed to have biological activity against a human retrovirus.

14. The sensor array of claim 10, wherein said synthetic oligonucleotide is an oligonucleotide designed to have biological activity against a human immunodeficiency virus.

15. The sensor array of claim 10, wherein said synthetic oligonucleotide is an oligonucleotide designed to have biological activity against a virus other than a human retrovirus.

16. The sensor array of claim 15, wherein said virus is influenza virus, Epstein-Barr virus, Respiratory Syncytial Virus, or cytomegalovirus.

17. A method of characterizing a sample comprising a plurality of target deletion sequence oligonucleotides, said method comprising the steps of:

isolating and labeling at least a substantial portion of the target deletion sequence oligonucleotide in said sample to obtain labeled target deletion sequence oligonucleotides;

contacting said labeled target deletion sequence oligonucleotides with the composition of claim 1 and allowing said labeled target deletion sequence oligonucleotides to undergo hybridization reactions with said probe oligonucleotide of said sensor arrays of said composition;

removing unhybridized oligonucleotides from said hybridization reactions; and

determining the amount of label bound to each probe of said sensor array, wherein the amount of said label bound to each probe of said sensor array is proportional to the amount of deletion sequence oligonucleotide specifically hybridizable thereto.

18. The method of claim 17, wherein said hybridization reactions are allowed to proceed to equilibrium.

19. The method of claim 17, wherein said hybridization reactions are allowed to proceed from about 1 to about 3 hours.

20. A method of characterizing a mixture of target deletion sequence oligonucleotides of length n-1 derived from a synthetic oligonucleotide of length n, said method comprising the step of contacting said sample with the composition of claim 10.

21. A method of characterizing a sample comprising a mixture of deletion sequence oligonucleotides of length n-1 derived from a synthetic oligonucleotide of length n, said method comprising the steps of:

isolating and labeling at least a substantial portion of the target deletion sequence oligonucleotide of length n-1 in said sample to obtain labeled target deletion sequence n-1 oligonucleotides;

contacting said labeled target deletion sequence oligonucleotides with the composition of claim 10 and allowing said labeled target deletion sequence oligonucleotides to undergo hybridization reactions with said probe oligonucleotide of said sensor arrays of said composition;

removing unhybridized oligonucleotides from said hybridization reactions; and

determining the amount of label bound to each probe of said sensor array, wherein the amount of said label bound to each probe of said sensor array is proportional to the amount of deletion sequence oligonucleotide specifically hybridizable thereto.

22. The method of claim 21, wherein said hybridization reactions are allowed to proceed to equilibrium.

23. The method of claim 21, wherein said hybridization reactions are allowed to proceed from about 1 to about 3 hours.

24. The method of claim 17, wherein said probe oligonucleotides of said sensor arrays are selected from the group consisting of peptide nucleic acids, oligodeoxyribonucleotides, oligoribonucleotides, chimeric oligonucleotides, oligonucleotides having one or more modified linkages, oligonucleotides having one or more modified sugar residues and oligonucleotides having one or more modified nucleobases.

25. The method of claim 21, wherein said synthetic oligonucleotide is designed to modulate cellular adhesion or cellular proliferation.

26. The method of claim 21, wherein said synthetic oligonucleotide is designed to have biological activity against a eukaryotic pathogen.

27. The method of claim 21, wherein said synthetic oligonucleotide is designed to have biological activity against a human retrovirus.

28. The method of claim 27, wherein said synthetic oligonucleotide is designed to have biological activity against a human immunodeficiency virus.

29. The method of claim 21, wherein said synthetic oligonucleotide is an oligonucleotide designed to have biological activity against a virus other than a human retrovirus.

30. The method of claim 29, wherein said virus is influenza virus, Epstein-Barr virus, Respiratory Syncytial Virus, or cytomegalovirus.

31. A pharmaceutical composition comprising an oligonucleotide characterized by the method of claim 17.

32. The method of claim 24, wherein said probe oligonucleotides of said sensor arrays are oligonucleotides having one or more modified linkages.

33. The method of claim 24, wherein said probe oligonucleotides of said sensor arrays are oligonucleotides having one or more modified sugar residues.

34. The method of claim 33, wherein said modified sugar residues have a 2' modification.

35. The method of claim 24, wherein said probe oligonucleotides of said sensor arrays are oligonucleotides having one or more modified nucleobases.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(I) APPLICANT: Isis Pharmaceuticals, Inc, et al.

(ii) TITLE OF INVENTION: Compositions and Methods for the Identification and Quantitation of Deletion Sequence Oligonucleotides in Synthetic Oligonucleotide Preparations

(iii) NUMBER OF SEQUENCES: 162

(iv) CORRESPONDENCE ADDRESS:

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(C) CITY: Philadelphia

(D) STATE: PA

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(F) ZIP: 19103

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: DISKETTE, 3.5 INCH, 1.44 MB STORAGE

(B) COMPUTER: IBM PS/2

(C) OPERATING SYSTEM: PC-DOS

(D) SOFTWARE: WORDPERFECT 6.1

(vi) CURRENT APPLICATION DATA: Related to:

(A) APPLICATION NUMBER: 923,771

(B) FILING DATE: September 2, 1997

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: John W. Caldwell

(B) REGISTRATION NUMBER: 28,937

(C) REFERENCE/DOCKET NUMBER: ISIS-3188

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (215) 568-3100

(B) TELEFAX: (215) 568-3439

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: ISIS 2922
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:
GCGTTTGCTC TTCTTCTTGC G 21

(2) INFORMATION FOR SEQ ID NO: 2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: (*n*-1) target oligonucleotide D1
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:
GCGTTTGCTC TTCTTCTTGG 20

(2) INFORMATION FOR SEQ ID NO: 3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:

(D) OTHER INFORMATION: (*n*-1) target oligonucleotide D2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GCGTTTGCTC TTCTTCTTCG 20

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 bases

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: (*n*-1) target oligonucleotide D3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GCGTTTGCTC TTCTTCTGCG 20

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 bases

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: (*n*-1) target oligonucleotide D4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GCGTTTGCTC TTCTTTTGCG 20

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 bases

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: (*n*-1) target oligonucleotide D5
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:
GCGTTTGCTC TTCTCTTGCG 20

(2) INFORMATION FOR SEQ ID NO: 7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: (*n*-1) target oligonucleotide D6
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:
GCGTTTGCTC TTTTCTTGCG 20

(2) INFORMATION FOR SEQ ID NO: 8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: (*n*-1) target oligonucleotide D7
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:
GCGTTTGCTC TCTTCTTGCG 20

(2) INFORMATION FOR SEQ ID NO: 9:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: (*n*-1) target oligonucleotide D8
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:
GCGTTTGCTT TCTTCTTGCG 20

(2) INFORMATION FOR SEQ ID NO: 10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: (*n*-1) target oligonucleotide D9
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:
GCGTTTGCCT TCTTCTTGCG 20

(2) INFORMATION FOR SEQ ID NO: 11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: (*n*-1) target oligonucleotide D10
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:
GCGTTTGTCT TCTTCTTGCG 20

(2) INFORMATION FOR SEQ ID NO: 12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: (*n*-1) target oligonucleotide D11
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:
GCGTTTCTCT TCTTCTTGCG 20

(2) INFORMATION FOR SEQ ID NO: 13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: (*n*-1) target oligonucleotide D12
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:
GCGTTGCTCT TCTTCTTGCG 20

(2) INFORMATION FOR SEQ ID NO: 14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: (*n*-1) target oligonucleotide

D13

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GCTTTGCTCT TCTTCTTGCG 20

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 bases

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: (n-1) target oligonucleotide D14

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GGTTTGCTCT TCTTCTTGCG 20

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 bases

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: probe for (n-1) target oligonucleotide D1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

TTTTTTTTTT TTTTTCCAAG AAGAAGA 27

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 bases

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: probe for (n-1) target oligonucleotide D2
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:
TTTTTTTTTT TTTTTCGAAG AAGAAGA 27

(2) INFORMATION FOR SEQ ID NO: 18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: probe for (n-1) target oligonucleotide D3
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:
TTTTTTTTTT TTTTTCGCAG AAGAAGA 27

(2) INFORMATION FOR SEQ ID NO: 19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: probe for (n-1) target oligonucleotide D4
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:
TTTTTTTTTT TTTTTCGCAA AAGAAGA 27

(2) INFORMATION FOR SEQ ID NO: 20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 bases

(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: probe for (n-1) target oligonucleotide D5
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:
TTTTTTTTTT TTTTTGCAAG AGAAGAG 27

(2) INFORMATION FOR SEQ ID NO: 21:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: probe for (n-1) target oligonucleotide D6
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:
TTTTTTTTTT TTTTTCAAGA AAAGAGC 27

(2) INFORMATION FOR SEQ ID NO: 22:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: probe for (n-1) target oligonucleotide D7
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:
TTTTTTTTTT TTTTTAGAAG AGAGCAA 27

(2) INFORMATION FOR SEQ ID NO: 23:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: probe for (*n*-1) target oligonucleotide D8
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:
TTTTTTTTTT TTTTTGAAGA AAGCAAA 27

(2) INFORMATION FOR SEQ ID NO: 24:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: probe for (*n*-1) target oligonucleotide D9
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:
TTTTTTTTTT TTTTTAAGAA GGCAAAC 27

(2) INFORMATION FOR SEQ ID NO: 25:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: probe for (*n*-1) target oligonucleotide D10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:
TTTTTTTTTT TTTTTAGAAG ACAAACG 27

(2) INFORMATION FOR SEQ ID NO: 26:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: probe for (n-1) target oligonucleotide D11
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:
TTTTTTTTTT TTTTTGAAGA GAAACGC 27

(2) INFORMATION FOR SEQ ID NO: 27:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: probe for (n-1) target oligonucleotide D12
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:
TTTTTTTTTT TTTTTGAAGA GCAACGC 27

(2) INFORMATION FOR SEQ ID NO: 28:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No

(ix) FEATURE:
(D) OTHER INFORMATION: probe for (n-1) target oligonucleotide D13
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:
TTTTTTTTTT TTTTTGAAGA GCAAAGC 27

(2) INFORMATION FOR SEQ ID NO: 29:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: probe for (n-1) target oligonucleotide D14
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:
TTTTTTTTTT TTTTTGAAGA GCAAACC 27

(2) INFORMATION FOR SEQ ID NO: 30:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 bases
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: probe for (n-1) target oligonucleotide D9
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:
TTTTTTTTTT TTTTTGAAGG CAA 23

(2) INFORMATION FOR SEQ ID NO: 31:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: ISIS 2302
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: US 5591623 (SEQ ID NO:22)
(I) FILING DATE: 21-JAN-1993
(J) PUBLICATION DATE: 07-JAN-1997
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:
GCCCAAGCTG GCATCCGTCA 20

(2) INFORMATION FOR SEQ ID NO: 32:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: GM1595
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: US 5580969 (SEQ ID NO:11)
(I) FILING DATE: 12-OCT-1993
(J) PUBLICATION DATE: 12-DEC-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:
AGCCATAGCG AGGCTGAGGT T 21

(2) INFORMATION FOR SEQ ID NO: 33:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: ISIS 5847

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: US 5591623 (SEQ ID NO:72)
(I) FILING DATE: 21-JAN-1993
(J) PUBLICATION DATE: 07-JAN-1997
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:
AACATCTCCG TACCATGCCA 20

(2) INFORMATION FOR SEQ ID NO: 34:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: GM1535
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: US 5596090 (SEQ ID NO:3)
(I) FILING DATE: 12-OCT-1993
(J) PUBLICATION DATE: 21-JAN-1997
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:
CCCAGGCATT TTAAGTTGCT G 21

(2) INFORMATION FOR SEQ ID NO: 35:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: GM1515
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: US 5585479 (SEQ ID NO:1)
(I) FILING DATE: 12-OCT-1993
(J) PUBLICATION DATE: 17-DEC-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

GTTTAAGGCA GCATCCTAAG A 21

(2) INFORMATION FOR SEQ ID NO: 36:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: GM1516
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: US 5585479 (SEQ ID NO:2)
(I) FILING DATE: 12-OCT-1993
(J) PUBLICATION DATE: 17-DEC-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:
TCACCCAAAG GTTTAGGCTT G 21

(2) INFORMATION FOR SEQ ID NO: 37:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: GM1517
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: US 5585479 (SEQ ID NO:3)
(I) FILING DATE: 12-OCT-1993
(J) PUBLICATION DATE: 17-DEC-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:
GCAATCATGA CTTCAAGAGT T 21

(2) INFORMATION FOR SEQ ID NO: 38:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs

(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to c-myb mRNA; a.k.a. "MYB-AS"
(x) PUBLICATION INFORMATION:
(A) AUTHORS: Calabretta, Bruno, et al.
(B) TITLE: Inhibition of Protooncogene Expression in Leukemic Cells: An Antisense Approach
(C) JOURNAL: Antisense Research and Applications, Crooke, S.T., et al., eds., CRC Press, Boca Raton
(D) VOLUME: Chapter 31
(F) PAGES: 535-545
(G) DATE: 1993
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:
GTGCCGGGGT CTTCGGGC 18

(2) INFORMATION FOR SEQ ID NO: 39:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to mammalian DNA methyl transferase
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/15378 (SEQ ID NO:1)
(I) FILING DATE: 30-NOV-1994
(J) PUBLICATION DATE: 08-JUN-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:
CATCTGCCAT TCCCACTCTA 20

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to mammalian DNA methyl transferase
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/15378 (SEQ ID NO:2)
(I) FILING DATE: 30-NOV-1994
(J) PUBLICATION DATE: 08-JUN-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:
TTGGCATCTG CCATTCCCAC TCTA 24

(2) INFORMATION FOR SEQ ID NO: 41:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Vascular Endothelial Growth factor (VEGF)
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/04142 (SEQ ID NO:1)
(I) FILING DATE: 26-JUL-1994
(J) PUBLICATION DATE: 09-FEB-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:
CATGGTTTCG GAGGGCGTC 19

(2) INFORMATION FOR SEQ ID NO: 42:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Vascular Endothelial Growth factor (VEGF)
(x) PUBLICATION INFORMATION:
(A) AUTHORS: Robinson, G.S., et al.
(B) TITLE: Oligodeoxynucleotides inhibit retinal neovascularization in a murine model of proliferative retinopathy (SEQ ID NO: M3)
(C) JOURNAL: The Proceedings of the National Academy of Sciences (U.S.A.)
(D) VOLUME: 93
(F) PAGES: 4851-4856
(G) DATE: MAY-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:
TCGCGCTCCC TCTCTCCGGC 20

(2) INFORMATION FOR SEQ ID NO: 43:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Vascular Endothelial Growth factor (VEGF)
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/04142 (SEQ ID NO: 4)
(I) FILING DATE: 26-JUL-1994
(J) PUBLICATION DATE: 09-FEB-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:
CACCCAAGAG AGCAGAAAGT 20

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Vascular Endothelial Growth factor (VEGF)
(x) PUBLICATION INFORMATION:
(A) AUTHORS: Nomura, M., et al.
(B) TITLE: Possible Participation of Autocrine and Paracrine Vascular Endothelial Growth factors in Hypoxia-induced Proliferation of Endothelial Cells and Pericytes
(C) JOURNAL: The Journal of Biological Chemistry
(D) VOLUME: 270
(E) ISSUE 47
(F) PAGES: 28316-28324
(G) DATE: 24-NOV-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:
CCCAAGACAG CAGAAAGTTC AT 22

(2) INFORMATION FOR SEQ ID NO: 45:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Vascular Endothelial Growth factor (VEGF)
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/04142 (SEQ ID NO: 5)
(I) FILING DATE: 26-JUL-1994
(J) PUBLICATION DATE: 09-FEB-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:
TCGTGGGTGC AGCCTGGGAC 20

(2) INFORMATION FOR SEQ ID NO: 46:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Vascular Endothelial Growth factor (VEGF)
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/04142 (SEQ ID NO:11)
(I) FILING DATE: 26-JUL-1994
(J) PUBLICATION DATE: 09-FEB-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:
CTGCCCGGCT CACCGCCTCG G 21

(2) INFORMATION FOR SEQ ID NO: 47:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Vascular Endothelial Growth factor (VEGF)
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/04142 (SEQ ID NO: 12)
(I) FILING DATE: 26-JUL-1994
(J) PUBLICATION DATE: 09-FEB-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:
CATGGTTTCG GAGGCCCGA 19

(2) INFORMATION FOR SEQ ID NO: 48:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Vascular Endothelial Growth factor (VEGF)
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/04142 (SEQ ID NO: 13)
(I) FILING DATE: 26-JUL-1994
(J) PUBLICATION DATE: 09-FEB-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:
CACCCAAGAC AGCAGAAAGT 20

(2) INFORMATION FOR SEQ ID NO: 49:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Vascular Endothelial Growth factor (VEGF)
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/04142 (SEQ ID NO:17)
(I) FILING DATE: 26-JUL-1994
(J) PUBLICATION DATE: 09-FEB-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:
CCATGGGTGC AGCCTGGGAC 20

(2) INFORMATION FOR SEQ ID NO: 50:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Vascular Endothelial Growth factor (VEGF)
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/04142 (SEQ ID NO: 17)
(I) FILING DATE: 26-JUL-1994
(J) PUBLICATION DATE: 09-FEB-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:
CCATGGGTGC AGCCTGGGAC 20

(2) INFORMATION FOR SEQ ID NO: 51:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to beta/A4 peptide
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO:1)
(I) FILING DATE: 28-SEP-1994
(J) PUBLICATION DATE: 06-APR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:
CCTCTCTGTT TAAAACTTTA TCCAT 25

(2) INFORMATION FOR SEQ ID NO: 52:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes

(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to beta/A4 peptide
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO:2)
(I) FILING DATE: 28-SEP-1994
(J) PUBLICATION DATE: 06-APR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:
TTCATATCCT GAGTCATGTC G 21

(2) INFORMATION FOR SEQ ID NO: 53:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to beta/A4 peptide
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO:3)
(I) FILING DATE: 28-SEP-1994
(J) PUBLICATION DATE: 06-APR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:
GTCCCAGCGC TACGACGGGC CAAA 24

(2) INFORMATION FOR SEQ ID NO: 54:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to beta/A4 peptide
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO:4)
(I) FILING DATE: 28-SEP-1994

(J) PUBLICATION DATE: 06-APR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

GTCCCAGCGC TAC 13

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to beta/A4 peptide

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO:5)

(I) FILING DATE: 28-SEP-1994

(J) PUBLICATION DATE: 06-APR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

TACGACGGGC CAAA 14

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to beta/A4 peptide

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO:6)

(I) FILING DATE: 28-SEP-1994

(J) PUBLICATION DATE: 06-APR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

GTCCCAGCGC TACGACGGGC C 21

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to beta/A4 peptide
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO:7)
(I) FILING DATE: 28-SEP-1994
(J) PUBLICATION DATE: 06-APR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:
GTCCCAGCGC TACGACGG 18

(2) INFORMATION FOR SEQ ID NO: 58:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to beta/A4 peptide
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO:8)
(I) FILING DATE: 28-SEP-1994
(J) PUBLICATION DATE: 06-APR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:
GTCCCAGCGC TACGA 15

(2) INFORMATION FOR SEQ ID NO: 59:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to beta/A4 peptide
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO:9)
(I) FILING DATE: 28-SEP-1994
(J) PUBLICATION DATE: 06-APR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:
CCAGCGCTAC GACGGGCCAA A 21

(2) INFORMATION FOR SEQ ID NO: 60:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to beta/A4 peptide
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO:10)
(I) FILING DATE: 28-SEP-1994
(J) PUBLICATION DATE: 06-APR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:
GCGCTACGAC GGGCCAAA 18

(2) INFORMATION FOR SEQ ID NO: 61:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to beta/A4 peptide
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO:11)

(I) FILING DATE: 28-SEP-1994
(J) PUBLICATION DATE: 06-APR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:
CTACGACGGG CCAAA 15

(2) INFORMATION FOR SEQ ID NO: 62:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to beta/A4 peptide
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO:15)
(I) FILING DATE: 28-SEP-1994
(J) PUBLICATION DATE: 06-APR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:
AAACCGGGCA GCATCGCGAC CCTG 24

(2) INFORMATION FOR SEQ ID NO: 63:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to beta-globin; a.k.a. "5'ss"
(x) PUBLICATION INFORMATION:
(A) AUTHORS: Sierakowska, H., et al.
(B) TITLE: Repair of thalassemic human β-globin in mammalian cells by antisense oligonucleotides
(C) JOURNAL: The Proceedings of the National Academy

of Sciences (U.S.A.)
(D) VOLUME: 93
(F) PAGES: 12840-12844
(G) DATE: 12-NOV-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:
GCUAUUACCU UAACCCAG 18

(2) INFORMATION FOR SEQ ID NO: 64:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to beta-globin; a.k.a. "3'ss"
(x) PUBLICATION INFORMATION:
(A) AUTHORS: Sierakowska, H., et al.
(B) TITLE: Repair of thalassemic human β-globin in mammalian cells by antisense oligonucleotides
(C) JOURNAL: The Proceedings of the National Academy of Sciences (U.S.A.)
(D) VOLUME: 93
(F) PAGES: 12840-12844
(G) DATE: 12-NOV-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:
CAUUAUUGCC CUGAAAG 17

(2) INFORMATION FOR SEQ ID NO: 65:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes

(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to malarial agents; a.k.a. "PSI"
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 93/13740
(I) FILING DATE: 31-DEC-1991
(J) PUBLICATION DATE: 22-JUL-1993
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:
TAAAAAGAAT ATGATCTTCA T 21

(2) INFORMATION FOR SEQ ID NO: 66:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to malarial agents; a.k.a. "PSII"
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 93/13740 (SEQ ID NO: PSII)
(I) FILING DATE: 31-DEC-1991
(J) PUBLICATION DATE: 22-JUL-1993
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:
AGCAACTGAG CCACCTGA 18

(2) INFORMATION FOR SEQ ID NO: 67:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to malarial agents; a.k.a. "PSIII"

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 93/13740
(I) FILING DATE: 31-DEC-1991
(J) PUBLICATION DATE: 22-JUL-1993
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:
GTCGCAGACT TGTTCCATCA T 21

(2) INFORMATION FOR SEQ ID NO: 68:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to malarial agents; a.k.a. "RI"
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 93/13740
(I) FILING DATE: 31-DEC-1991
(J) PUBLICATION DATE: 22-JUL-1993
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:
CTTGGCAGCT GCGCGTGACA T 21

(2) INFORMATION FOR SEQ ID NO: 69:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to scistosome worms
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/33759 (SEQ ID NO:1)
(I) FILING DATE: 30-MAY-1995
(J) PUBLICATION DATE: 14-DEC-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:
GCCATAGGGG GCAGGGAAGG C 21

(2) INFORMATION FOR SEQ ID NO: 70:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HTLV-III
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 87/07300 (SEQ ID NO:A)
(I) FILING DATE: 22-MAY-1987
(J) PUBLICATION DATE: 03-DEC-1987
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:
CTGCTAGAGA TT 12

(2) INFORMATION FOR SEQ ID NO: 71:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HTLV-III
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 87/07300 (SEQ ID NO:B)
(I) FILING DATE: 22-MAY-1987
(J) PUBLICATION DATE: 03-DEC-1987
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:
CTGCTAGAGA TTTTCCACAC 20

(2) INFORMATION FOR SEQ ID NO: 72:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HTLV-III
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 87/07300 (SEQ ID NO:C)
(I) FILING DATE: 22-MAY-1987
(J) PUBLICATION DATE: 03-DEC-1987
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:
TTCAAGTCCC TGTTCGGGCG CCAAA 25

(2) INFORMATION FOR SEQ ID NO: 73:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HTLV-III
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 87/07300 (SEQ ID NO:D)
(I) FILING DATE: 22-MAY-1987
(J) PUBLICATION DATE: 03-DEC-1987
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:
GCGTACTCAC CAGTCGCCGC 20

(2) INFORMATION FOR SEQ ID NO: 74:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes

(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HTLV-III
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 87/07300 (SEQ ID NO:E)
(I) FILING DATE: 22-MAY-1987
(J) PUBLICATION DATE: 03-DEC-1987
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:
CTGCTAGAGA TTAA 14

(2) INFORMATION FOR SEQ ID NO: 75:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HTLV-III
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 87/07300 (SEQ ID NO:F)
(I) FILING DATE: 22-MAY-1987
(J) PUBLICATION DATE: 03-DEC-1987
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:
ACACCCAATT CTGAAAATGG 20

(2) INFORMATION FOR SEQ ID NO: 76:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV-1
(x) PUBLICATION INFORMATION:
(A) AUTHORS: Agrawal, Sudhir
Tang, Jin Yan

(B) TITLE: GEM 91-An Antisense Oligonucleotide Phosphorothioate as a Therapeutic Agent for AIDS
(C) JOURNAL: Antisense Research and Development
(D) VOLUME: 2
(E) ISSUE: 6
(F) PAGES: 261-266
(G) DATE: Winter-1992
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO:1)
(I) FILING DATE: 04-OCT-1993
(J) PUBLICATION DATE: 14-APR-1994
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:
CTCTCGCACC CATCTCTCTC CTTCT 25

(2) INFORMATION FOR SEQ ID NO: 77:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV-1
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO:2)
(I) FILING DATE: 04-OCT-1993
(J) PUBLICATION DATE: 14-APR-1994
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 77:
CTCTCGCACC CATCTCTCTC CTTCTA 26

(2) INFORMATION FOR SEQ ID NO: 78:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes

(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV-1
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO:3)
(I) FILING DATE: 04-OCT-1993
(J) PUBLICATION DATE: 14-APR-1994
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:
GCTCTCGCAC CCATCTCTCT CCTTCT 26

(2) INFORMATION FOR SEQ ID NO: 79:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV-1
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO:4)
(I) FILING DATE: 04-OCT-1993
(J) PUBLICATION DATE: 14-APR-1994
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 79:
GCTCTCGCAC CCATCTCTCT CCTTCTA 27

(2) INFORMATION FOR SEQ ID NO: 80:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV-1
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO:5)
(I) FILING DATE: 04-OCT-1993

(J) PUBLICATION DATE: 14-APR-1994

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 80:

GCTCTCGCAC CCATCTCTCT CCTTCTAG 28

(2) INFORMATION FOR SEQ ID NO: 81:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to HIV-1

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO:6)

(I) FILING DATE: 04-OCT-1993

(J) PUBLICATION DATE: 14-APR-1994

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 81:

CGCTCTCGCA CCCATCTCTC TCCTTCTA 28

(2) INFORMATION FOR SEQ ID NO: 82:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to HIV-1

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO:7)

(I) FILING DATE: 04-OCT-1993

(J) PUBLICATION DATE: 14-APR-1994

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 82:

CGCTCTCGCA CCCATCTCTC TCCTTCTAG 29

(2) INFORMATION FOR SEQ ID NO: 83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV-1
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO:8)
(I) FILING DATE: 04-OCT-1993
(J) PUBLICATION DATE: 14-APR-1994
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 83:
CGCTCTCGCA CCCATCTCTC TCCTTCTAGC 30

(2) INFORMATION FOR SEQ ID NO: 84:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV-1
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO:9)
(I) FILING DATE: 04-OCT-1993
(J) PUBLICATION DATE: 14-APR-1994
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 84:
ACGCTCTCGC ACCCATCTCT CTCCTTCTAG 30

(2) INFORMATION FOR SEQ ID NO: 85
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV-1
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO:10)
(I) FILING DATE: 04-OCT-1993
(J) PUBLICATION DATE: 14-APR-1994
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 85:
CTCGCACCCA TCTCTCTCCT 20

(2) INFORMATION FOR SEQ ID NO: 86:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV-1; a.k.a. "AR 177"
(x) PUBLICATION INFORMATION:
(A) AUTHORS: Bishop, J.S., et al.
(B) TITLE: Intramolecular G-quartet Motifs Confer Nuclease Resistance to a Potent Anti-HIV Oligonucleotide
(C) JOURNAL: The Journal of Biological Chemistry
(D) VOLUME: 271
(E) ISSUE: 10
(F) PAGES: 5698-5703
(G) DATE: 08-MAR-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 86:
GTGGTGGGTG GGTGGGT 17

(2) INFORMATION FOR SEQ ID NO: 87:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 87:
GCCTATTCTG CTATGTCGAC ACCCAA 26

(2) INFORMATION FOR SEQ ID NO: 88:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 88:
CTTCGGGCCT GTCGGGTCCC CTCGGG 26

(2) INFORMATION FOR SEQ ID NO: 89:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/03407
(I) FILING DATE: 19-JUL-1994
(J) PUBLICATION DATE: 02-FEB-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 89:
CTTCGGGCCT GTCGGGTCCC CTCGGG 26

(2) INFORMATION FOR SEQ ID NO: 90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 96/02557 (SEQ ID NO:3)
(I) FILING DATE: 14-JUL-1995
(J) PUBLICATION DATE: 01-FEB-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 90:
GCTGGTGATC CTTTCCATCC CTGTGG 26

(2) INFORMATION FOR SEQ ID NO: 91:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 96/02557 (SEQ ID NO:5)
(I) FILING DATE: 14-JUL-1995
(J) PUBLICATION DATE: 01-FEB-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 91:
CTACTACTCC TTGACTTTGG GGATTG 26

(2) INFORMATION FOR SEQ ID NO: 92:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 96/02557 (SEQ ID NO:6)
(I) FILING DATE: 14-JUL-1995
(J) PUBLICATION DATE: 01-FEB-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 92:
CCTCTGTTAG TAACATATCC TGCTTTTCC 29

(2) INFORMATION FOR SEQ ID NO: 93:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 96/02557 (SEQ ID NO:8)
(I) FILING DATE: 14-JUL-1995
(J) PUBLICATION DATE: 01-FEB-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 93:
GGTTGCTTCC TTCCTCTCTG GTACCC 26

(2) INFORMATION FOR SEQ ID NO: 94:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 96/02557 (SEQ ID NO:10)

(I) FILING DATE: 14-JUL-1995
(J) PUBLICATION DATE: 01-FEB-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 94:
CTAGCAGTGG CGCCCGAACA GGTTCGCCTG TTCGGGCGCC 40
A 41

(2) INFORMATION FOR SEQ ID NO: 95:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 96/02557 (SEQ ID NO:22)
(I) FILING DATE: 14-JUL-1995
(J) PUBLICATION DATE: 01-FEB-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 95:
CATCACCTGC CATCTGTTTT CCATAATCCC 30

(2) INFORMATION FOR SEQ ID NO: 96:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 96/02557 (SEQ ID NO:23)
(I) FILING DATE: 14-JUL-1995
(J) PUBLICATION DATE: 01-FEB-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 96:
CCTGTCTACT TGCCACACAA TCATCACCTG C 31

(2) INFORMATION FOR SEQ ID NO: 97:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 96/02557 (SEQ ID NO:25)
(I) FILING DATE: 14-JUL-1995
(J) PUBLICATION DATE: 01-FEB-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 97:
ACTATTGCTA TTATTATTGC TACTACTAAT 30

(2) INFORMATION FOR SEQ ID NO: 98:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/03406 (SEQ ID NO:1)
(I) FILING DATE: 19-JUL-1994
(J) PUBLICATION DATE: 02-FEB-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 98:
CTTCGGGCCT GTCGGGTCCC CTCGGG 26

(2) INFORMATION FOR SEQ ID NO: 99:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/03406 (SEQ ID NO:2)
(I) FILING DATE: 19-JUL-1994
(J) PUBLICATION DATE: 02-FEB-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 99:
CUUCGGGCCU GUCGGGUCC CUCGGG 26

(2) INFORMATION FOR SEQ ID NO: 100:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/03406 (SEQ ID NO:3)
(I) FILING DATE: 19-JUL-1994
(J) PUBLICATION DATE: 02-FEB-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 100:
GCCTGTCGGG TCCC 14

(2) INFORMATION FOR SEQ ID NO: 101:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/03406 (SEQ ID NO:4)
(I) FILING DATE: 19-JUL-1994
(J) PUBLICATION DATE: 02-FEB-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 101:
GCCUGUCGGG UCCC 14

(2) INFORMATION FOR SEQ ID NO: 102:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/03406 (SEQ ID NO:5)
(I) FILING DATE: 19-JUL-1994
(J) PUBLICATION DATE: 02-FEB-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 102:
CTTCGGGCCT GTCGGGTCCC CTCGGG 26

(2) INFORMATION FOR SEQ ID NO: 103:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/03406 (SEQ ID NO:6)
(I) FILING DATE: 19-JUL-1994
(J) PUBLICATION DATE: 02-FEB-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 103:

GCTGGTGATC CTTTCCATCC CTGTGG 26

(2) INFORMATION FOR SEQ ID NO: 104:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV; a.k.a. "ISIS 5320"
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: US 5523389
(I) FILING DATE: 28-SEP-1994
(J) PUBLICATION DATE: 04-JUN-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 190:
TTGGGGTT 8

(2) INFORMATION FOR SEQ ID NO: 105:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Hepatitis C Virus; a.k.a. "ISIS 6547"
(x) PUBLICATION INFORMATION:
(A) AUTHORS: Hanecak, R., et al.
(B) TITLE: Intramolecular G-quartet Motifs Confer Nuclease Resistance to a Potent Anti-HIV Oligonucleotide
(C) JOURNAL: Journal of Virology
(D) VOLUME: 70
(E) ISSUE: 8

(F) PAGES: 5203-5212

(G) DATE: 01-AUG-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 105:

GTGCTCATGG TGCACGGTCT 20

(2) INFORMATION FOR SEQ ID NO: 106:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to influenza virus

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 91/16902 (SEQ ID NO:1)

(I) FILING DATE: 29-APR-1991

(J) PUBLICATION DATE: 14-NOV-1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 106:

CATTCAAATG GTTTGCCTGC 20

(2) INFORMATION FOR SEQ ID NO: 107:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to influenza virus

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 91/16902 (SEQ ID NO:2)

(I) FILING DATE: 29-APR-1991

(J) PUBLICATION DATE: 14-NOV-1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 107:

GCAGGCAAAC CATTTGAATG 20

(2) INFORMATION FOR SEQ ID NO: 108:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to influenza virus

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 91/16902 (SEQ ID NO:3)

(I) FILING DATE: 29-APR-1991

(J) PUBLICATION DATE: 14-NOV-1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 108:

CCATAATCCC CTGCTTCTGC 20

(2) INFORMATION FOR SEQ ID NO: 109:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to influenza virus

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 91/16902 (SEQ ID NO:4)

(I) FILING DATE: 29-APR-1991

(J) PUBLICATION DATE: 14-NOV-1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 109:

GCAGAAGCAG GGGATTATGG 20

(2) INFORMATION FOR SEQ ID NO: 110:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to influenza virus
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 91/16902 (SEQ ID NO:5)
(I) FILING DATE: 29-APR-1991
(J) PUBLICATION DATE: 14-NOV-1991
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 110:
GCAGAAGCAG AGGATTATGG 20

(2) INFORMATION FOR SEQ ID NO: 111:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to influenza virus
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 91/16902 (SEQ ID NO:6)
(I) FILING DATE: 29-APR-1991
(J) PUBLICATION DATE: 14-NOV-1991
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 111:
GCATAAGCAG AGGATCATGG 20

(2) INFORMATION FOR SEQ ID NO: 112:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to influenza virus
(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 91/16902 (SEQ ID NO:7)
(I) FILING DATE: 29-APR-1991
(J) PUBLICATION DATE: 14-NOV-1991
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 112:
GGCAAGCTTT ATTGAGGCTT 20

(2) INFORMATION FOR SEQ ID NO: 113:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to influenza virus
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 91/16902 (SEQ ID NO:8)
(I) FILING DATE: 29-APR-1991
(J) PUBLICATION DATE: 14-NOV-1991
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 113:
ATCTTCATCA TCTGAGAGAT 20

(2) INFORMATION FOR SEQ ID NO: 114:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to influenza virus
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 91/16902 (SEQ ID NO:9)
(I) FILING DATE: 29-APR-1991
(J) PUBLICATION DATE: 14-NOV-1991
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 114:
CGTAAGCAAC AGTAGTCCTA 20

(2) INFORMATION FOR SEQ ID NO: 115:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to Epstein-Barr Virus

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/22554 (SEQ ID NO:1)

(I) FILING DATE: 17-FEB-1995

(J) PUBLICATION DATE: 24-AUG-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 115:

TTTGGGTCCA TCATCTTCAG CAAAG 25

(2) INFORMATION FOR SEQ ID NO: 116:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to Epstein-Barr Virus

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/22554 (SEQ ID NO:2)

(I) FILING DATE: 17-FEB-1995

(J) PUBLICATION DATE: 24-AUG-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 116:

CATCATCTTC AGCAAAGATA 20

(2) INFORMATION FOR SEQ ID NO: 117:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Epstein-Barr Virus
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/22554 (SEQ ID NO:3)
(I) FILING DATE: 17-FEB-1995
(J) PUBLICATION DATE: 24-AUG-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 117:
TCAGAAGTCG AGTTTGGGTC 20

(2) INFORMATION FOR SEQ ID NO: 118:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Respiratory Syncytial Virus
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/22553 (SEQ ID NO:1)
(I) FILING DATE: 17-FEB-1995
(J) PUBLICATION DATE: 24-AUG-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 118:
ACGCGAAAAA ATGCGTACAA 20

(2) INFORMATION FOR SEQ ID NO: 119:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Respiratory Syncytial Virus
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/22553 (SEQ ID NO:2)
(I) FILING DATE: 17-FEB-1995
(J) PUBLICATION DATE: 24-AUG-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 119:
TAAACCAAAA AAATGGGGCA 20

(2) INFORMATION FOR SEQ ID NO: 120:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Respiratory Syncytial Virus
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/22553 (SEQ ID NO:3)
(I) FILING DATE: 17-FEB-1995
(J) PUBLICATION DATE: 24-AUG-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 120:
AAATGGGGCA AATAAGAATT 20

(2) INFORMATION FOR SEQ ID NO: 121:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to Respiratory Syncytial Virus

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/22553 (SEQ ID NO:4)

(I) FILING DATE: 17-FEB-1995

(J) PUBLICATION DATE: 24-AUG-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 121:

AAAAATGGGG CAAATAAATC 20

(2) INFORMATION FOR SEQ ID NO: 122:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to cytomagalovirus intron-exon boundary of genes UL36 and UL37; a.k.a. "UL36ANTI" and "GEM 132"

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Pari, G.S., et al.

(B) TITLE: Potent Antiviral Activity of an Antisense Oligonucleotide Complementary to the Intron-Exon Boundary of Human Cytomegalovirus Genes UL36 and UL37

(C) JOURNAL: Antimicrobial Agents and Chemotherapy

(D) VOLUME: 39

(E) ISSUE: 5

(F) PAGES: 1157-1161

(G) DATE: MAY-1995

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/32213 (SEQ ID NO:1)

(I) FILING DATE: 19-MAY-1995

(J) PUBLICATION DATE: 30-NOV-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 122:

TGGGGCTTAC CTTGCGAACA 20

(2) INFORMATION FOR SEQ ID NO: 123:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to cytomagalovirus

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/32213 (SEQ ID NO:2)

(I) FILING DATE: 19-MAY-1995

(J) PUBLICATION DATE: 30-NOV-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 123:

GACGTGGGGC TTACCTTGCG 20

(2) INFORMATION FOR SEQ ID NO: 124:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to cytomagalovirus

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/32213 (SEQ ID NO:3)

(I) FILING DATE: 19-MAY-1995

(J) PUBLICATION DATE: 30-NOV-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 124:

TCTTCAACGA CGTGGGGCTT 20

(2) INFORMATION FOR SEQ ID NO: 125:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to cytomagalovirus
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/32213 (SEQ ID NO:4)
(I) FILING DATE: 19-MAY-1995
(J) PUBLICATION DATE: 30-NOV-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 125:
GACGCGTGGC ATGCTTGGTG T 21

(2) INFORMATION FOR SEQ ID NO: 126:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to cytomagalovirus
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/32213 (SEQ ID NO:5)
(I) FILING DATE: 19-MAY-1995
(J) PUBLICATION DATE: 30-NOV-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 126:
AGGTTGGGGT CGACGCGTGG C 21

(2) INFORMATION FOR SEQ ID NO: 127:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to cytomagalovirus
(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/32213 (SEQ ID NO:6)
(I) FILING DATE: 19-MAY-1995
(J) PUBLICATION DATE: 30-NOV-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 127:
GGCTGAGCGG TCATCCTCGG A 21

(2) INFORMATION FOR SEQ ID NO: 128:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to cytomagalovirus
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/32213 (SEQ ID NO:7)
(I) FILING DATE: 19-MAY-1995
(J) PUBLICATION DATE: 30-NOV-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 128:
CGGGACTCAC CGTCGTTCTG 20

(2) INFORMATION FOR SEQ ID NO: 129:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to cytomagalovirus
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/32213 (SEQ ID NO:8)
(I) FILING DATE: 19-MAY-1995
(J) PUBLICATION DATE: 30-NOV-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 129:
GGAGGAGAGC CTACAGACGG 20

(2) INFORMATION FOR SEQ ID NO: 130:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to cytomagalovirus
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/32213 (SEQ ID NO:9)
(I) FILING DATE: 19-MAY-1995
(J) PUBLICATION DATE: 30-NOV-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 130:
AGTAACGCAC CGTCGGTGCC 20

(2) INFORMATION FOR SEQ ID NO: 131:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to cytomegalovirus; a.k.a. "ISIS 2922"
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: US 5442049 (SEQ ID NO:22)
(I) FILING DATE: 25-JAN-1993
(J) PUBLICATION DATE: 15-AUG-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 131:
GCGTTTGCTC TTCTTCTTGC G 21

(2) INFORMATION FOR SEQ ID NO: 132:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs

(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to VEGF/VPF; a.k.a. "H3"
(x) PUBLICATION INFORMATION:
(A) AUTHORS: Smyth, A.P., et al.
(B) TITLE: Antisense Oligonucleotides Inhibit Vascular Endothelial Growth Factor/vascular Permeability Factor Expression in Normal Human Epidermal Keratinocytes Boundary of Human Cytomegalovirus Genes UL36 and UL37
(C) JOURNAL: The Journal of Investigative Dermatology
(D) VOLUME: 108
(E) ISSUE: 4
(F) PAGES: 523-526
(G) DATE: xx-APR-1997
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 132:
CACCCAAGAC AGCAGAAAG 19

(2) INFORMATION FOR SEQ ID NO: 133:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Vascular Endothelial Growth factor (VEGF); a.k.a. "Vm"
(x) PUBLICATION INFORMATION:
(A) AUTHORS: Robinson, G.S., et al.
(B) TITLE: Oligodeoxynucleotides inhibit retinal neovascularization in a murine model of

proliferative retinopathy
(C) JOURNAL: The Proceedings of the National Academy of Sciences (U.S.A.)
(D) VOLUME: 93
(F) PAGES: 4851-4856
(G) DATE: MAY-1996
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/04142 (SEQ ID NO:2)
(I) FILING DATE: 26-JUL-1994
(J) PUBLICATION DATE: 09-FEB-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 133:
CAGCCTGGCT CACCGCCTTG G 21

(2) INFORMATION FOR SEQ ID NO: 134:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to bcl-2 mRNA
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 1)
(I) FILING DATE: 20-SEP-1994
(J) PUBLICATION DATE: 30-MAR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 134:
CCCTTCCTAC CGCGTGCGAC 20

(2) INFORMATION FOR SEQ ID NO: 135:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to bcl-2 mRNA
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 3)
(I) FILING DATE: 20-SEP-1994
(J) PUBLICATION DATE: 30-MAR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 135:
CCTCCGACCC ATCCACGTAG 20

(2) INFORMATION FOR SEQ ID NO: 136:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to bcl-2 mRNA
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 5)
(I) FILING DATE: 20-SEP-1994
(J) PUBLICATION DATE: 30-MAR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 136:
GTTGACGTCC TACGGAAACA 20

(2) INFORMATION FOR SEQ ID NO: 137:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to bcl-2 mRNA
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 8)
(I) FILING DATE: 20-SEP-1994
(J) PUBLICATION DATE: 30-MAR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 137:
CGCGTGCGAC CCTCTTG 17

(2) INFORMATION FOR SEQ ID NO: 138:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to bcl-2 mRNA
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 9)
(I) FILING DATE: 20-SEP-1994
(J) PUBLICATION DATE: 30-MAR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 138:
TCCTACCGCG TGCGACC 17

(2) INFORMATION FOR SEQ ID NO: 139:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to bcl-2 mRNA
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 10)
(I) FILING DATE: 20-SEP-1994
(J) PUBLICATION DATE: 30-MAR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 139:
TCCTACCGCG TGCGACC 17

(2) INFORMATION FOR SEQ ID NO: 140:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to bcl-2 mRNA
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 11)
(I) FILING DATE: 20-SEP-1994
(J) PUBLICATION DATE: 30-MAR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 140:
CCTTCCTACC GCGTGCG 17

(2) INFORMATION FOR SEQ ID NO: 141:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to bcl-2 mRNA
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 12)
(I) FILING DATE: 20-SEP-1994
(J) PUBLICATION DATE: 30-MAR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 141
GACCCTTCCT ACCGCGT 17

(2) INFORMATION FOR SEQ ID NO: 142:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to bcl-2 mRNA
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 13)
(I) FILING DATE: 20-SEP-1994
(J) PUBLICATION DAT E: 30-MAR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 142:
GGAGACCCTT CCTACCG 17

(2) INFORMATION FOR SEQ ID NO: 143:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to bcl-2 mRNA
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 14)
(I) FILING DATE: 20-SEP-1994
(J) PUBLICATION DATE: 30-MAR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 143:
GCGGCGGCAG CGCGG 15

(2) INFORMATION FOR SEQ ID NO: 144:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to bcl-2 mRNA
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 15)
(I) FILING DATE: 20-SEP-1994
(J) PUBLICATION DATE: 30-MAR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 144:
CGGCGGGGCG ACGGA 15

(2) INFORMATION FOR SEQ ID NO: 145:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to bcl-2 mRNA
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 16)
(I) FILING DATE: 20-SEP-1994
(J) PUBLICATION DATE: 30-MAR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 145:
CGGGAGCGCG GCGGGC 16

(2) INFORMATION FOR SEQ ID NO: 146:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to bcl-2 mRNA; a.k.a. "BCL-2"
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO:17)
(I) FILING DATE: 20-SEP-1994
(J) PUBLICATION DATE: 30-MAR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 146:
TCTCCCAGCG TGCGCCAT 18

(2) INFORMATION FOR SEQ ID NO: 147:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to bcl/abl mRNA
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 92/02641
(I) FILING DATE: 09-AUG-1991
(J) PUBLICATION DATE: 20-FEB-1992
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 147:
GGCGTTTTGA ACTCTGCTT 19

(2) INFORMATION FOR SEQ ID NO: 148:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to several isoforms of PKC; a.k.a. "$\text{oligo}_{\text{anti-PKC}\alpha}$"
(x) PUBLICATION INFORMATION:
(A) AUTHORS: Shih, M., et al.
(B) TITLE: Oligodeoxynucleotides antisense to mRNA encoding protein kinase A, protein kinase C and β-adrenergic receptor kinase reveal distinctive cell-type-specific roles in agonist-induced desensitization
(C) JOURNAL: The Proceedings of the National Academy of Sciences (U.S.A.)
(D) VOLUME: 91
(F) PAGES: 12193-12197

(G) DATE: 06-DEC-1994

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 148:

AAGGTGGGCT GCTTGAAGAA 20

(2) INFORMATION FOR SEQ ID NO: 149:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to ζ-Protein Kinase C gene

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 93/20101 (SEQ ID NO:14)

(I) FILING DATE: 02-APR-1993

(J) PUBLICATION DATE: 14-OCT-199

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 149:

GGTCCTGCTG GGCAT 15

(2) INFORMATION FOR SEQ ID NO: 150:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to α-Protein Kinase C gene; a.k.a. "ISIS 3521"

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/02069 (SEQ ID NO:2)

(I) FILING DATE: 08-JUL-1994

(J) PUBLICATION DATE: 19-JAN-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 150:

GTTCTCGCTG GTGAGTTTCA 20

(2) INFORMATION FOR SEQ ID NO: 151:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 97/11171 (SEQ ID NO:1)
(I) FILING DATE: 19-SEP-1996
(J) PUBLICATION DATE: 27-MAR-1997
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 151:
GCGTGCCTCC TCACTGGC 18

(2) INFORMATION FOR SEQ ID NO: 152:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 97/11171 (SEQ ID NO:4)
(I) FILING DATE: 19-SEP-1996
(J) PUBLICATION DATE: 27-MAR-1997
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 152:
GCGUGCCTCC TCACUGGC 18

(2) INFORMATION FOR SEQ ID NO: 153:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 97/11171 (SEQ ID NO:6)
(I) FILING DATE: 19-SEP-1996
(J) PUBLICATION DATE: 27-MAR-1997
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 153:
GCGTGCCUCC UCACTGGC 18

(2) INFORMATION FOR SEQ ID NO: 154:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to βARK1 and βARK2; a.k.a. as "$oligo_{anti\text{-}\beta ARK2}$"
(x) PUBLICATION INFORMATION:
(A) AUTHORS: Shih, M., et al.
(B) TITLE: Oligodeoxynucleotides antisense to mRNA encoding protein kinase A, protein kinase C and β-adrenergic receptor kinase reveal distinctive cell-type-specific roles in agonist-induced desensitization
(C) JOURNAL: The Proceedings of the National Academy of Sciences (U.S.A.)
(D) VOLUME: 91
(F) PAGES: 12193-12197
(G) DATE: 06-DEC-1994
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 154:
ACCGCCTCCA GGTCCGCCAT 20

(2) INFORMATION FOR SEQ ID NO: 155:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Ha-ras Gene; a.k.a. "ISIS 2503"
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: US 5576208 (SEQ ID NO:2)
(I) FILING DATE: 26-AUG-1994
(J) PUBLICATION DATE: 19-NOV-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 155:
TCCGTCATCG CTCCTCAGGG 20

(2) INFORMATION FOR SEQ ID NO: 156:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Multi-drug Resistance-1 (MDR-1) gene
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 96/02556 (SEQ ID NO:1)
(I) FILING DATE: 18-JUL-1995
(J) PUBLICATION DATE: 01-FEB-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 156:
TGTGCTCTTC CCACAGCCAC TG 22

(2) INFORMATION FOR SEQ ID NO: 157:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes

(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Multi-drug Resistance-1 (MDR-1) gene
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 96/02556 (SEQ ID NO:2)
(I) FILING DATE: 18-JUL-1995
(J) PUBLICATION DATE: 01-FEB-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 157:
TGTGCTCTTC CCACAGCCAC 20

(2) INFORMATION FOR SEQ ID NO: 158:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Multi-drug Resistance-1 (MDR-1) gene
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 96/02556 (SEQ ID NO:3)
(I) FILING DATE: 18-JUL-1995
(J) PUBLICATION DATE: 01-FEB-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 158:
GTGCTCTTCC CACAGCCACT 20

(2) INFORMATION FOR SEQ ID NO: 159:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Multi-drug Resistance-1 (MDR-1) gene

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 96/02556 (SEQ ID NO:4)
(I) FILING DATE: 18-JUL-1995
(J) PUBLICATION DATE: 01-FEB-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 159:
TGCTCTTCCC ACAGCCACTG 20

(2) INFORMATION FOR SEQ ID NO: 160:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to multidrug resistance-associated protein (MRP) gene; a.k.a. "ISIS 7597"
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: US 5510239 (SEQ ID NO:8)
(I) FILING DATE: 18-OCT-1993
(J) PUBLICATION DATE: 23-APR-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 160:
TGCTGTTCGT GCCCCCGCCG 20

(2) INFORMATION FOR SEQ ID NO: 161:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to *A-raf* gene; a.k.a. "ISIS 9069"
(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: US 5563255 (SEQ ID NO:37)
(I) FILING DATE: 31-MAY-1994
(J) PUBLICATION DATE: 08-OCT-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 161:
CTAAGGCACA AGGCGGGCTG 20

(2) INFORMATION FOR SEQ ID NO: 162:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to c-raf kinase Gene; a.k.a. "ISIS 5132"
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: US 5563255 (SEQ ID NO:8)
(I) FILING DATE: 05-31-1994
(J) PUBLICATION DATE: 08-OCT-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 162:
TCCCGCCTGT GACATGCATT 20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/18084

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C12Q 1/68
US CL :435/6
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS, BIOSIS, MEDLINE, BIOTECH ABS, WPI
covering terms: array, hybridization, deletion, sequence

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US, 5,632,957 A (HELLER et al.) 27 May 1997, see especially the abstract and claims 1-60.	1-10 and 17 ----------- 11-16
X -- Y	US 5,252,743 A (BARRETT et al.) 12 October 1993, see especially the abstract and the summary of the invention in column 2, line 36, through column 3, line 6.	1-10 and 17 ----------- 11-16
X -- Y	SOUTHERN et al., Analyzing and Comparing Nucleic Acid Sequences by Hybridization to Arrays of Oligonucleotides: Evaluation Using Experimental Models. GENOMICS. 1992, Volume 13, pages 1008-1017, see especially the entire disclosure.	1-10 and 17 ----------- 11-16

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 SEPTEMBER 1998	28 OCT 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ARDIN MARSCHEL Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/18084

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 86/05518 A1 (SUMMERTON et al.) 25 September 1986, see especially the abstract and claims 1-15.	1-10 and 17 ---------- 11-16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/18084

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

422/50, 68.1; 435/6; 436/501; 536/23.1,24.1,24.3,24.31,24.32,24.33; 935/77,78

Form PCT/ISA/210 (extra sheet)(July 1992)*